

From Bench to Bedside:
Understanding Multiple Dimensions of Pediatric HIV in Kenya

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Abstract

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Introduction: Globally, over 3.3 million children are infected with HIV. Despite significant progress, there is a need to better understand mechanisms for transmission and progression of HIV in children. In addition, among children receiving HIV care, it is important to determine the best ways to inform them about their diagnosis.

Methods: Consistent with the University of Washington Public Health Genetics PhD requirement of including both genetic epidemiology and social/cultural domains, my PhD dissertation addresses two projects of relevance to children with HIV. The aim of the first project is to determine the role of selected genetic mechanisms influencing pediatric HIV acquisition and progression. The aim of the second project is to determine how, when and what healthcare providers decide to tell HIV-infected children about their diagnosis.

For project 1, I used genetic epidemiology methods to evaluate the role of variations in innate immune system genes on infant HIV acquisition and progression in a Kenyan mother-to-child transmission (MTCT) cohort. Specifically, I genotyped infants from this cohort for 6 candidate and 118 haplotype-tagging polymorphisms in *TLRs* 2, 3, 4, 7, 8, and 9, *MyD88* and *TIRAP*, and 144 ancestral informative markers. Cox proportional hazards and linear regression were performed to assess *TLR* polymorphism associations with HIV acquisition, peak HIV RNA levels, and infant mortality. Sex-stratified analyses of *TLR7* and *TLR8* were conducted due to their X-chromosome location and Bonferroni methods were used to account for multiple comparisons.

For project 2, I used qualitative methods to analyze transcripts from semi-structured interviews conducted with 21 healthcare providers caring for HIV-infected children from 5 clinics in Kenya. Interview transcripts were systematically coded and conceptually analyzed using modified grounded theory and thematic network analysis approaches. Resulting themes were identified related to the disclosure processes, ethical and practical rationale for different approaches, and challenges or barriers to disclosure.

Results: For project 1, I found that *TLR* variants influenced HIV acquisition and progression. Infants with the *TLR9* 1635A (rs352140) variant were more likely to acquire HIV by 1 month of age (HR=1.81, 95% CI: 1.05, 3.14; p=0.033) and 12 months of age (HR=1.62, 95% CI: 1.01, 2.60; p=0.044). I also found that among 56 infants infected by 1 month of age, the *TLR9* 1635A allele was associated with a decrease in peak viral load (-0.58 log₁₀ c/ml, 95% CI: -0.95, -0.22; p=0.002) whereas female infants with the *TLR8* 1G (rs3764880) variant had increased peak viral load (0.78 log₁₀ c/ml, 95% CI: 0.35, 1.21; p<0.001). I also found that among female infants infected at less than 1 year of age, infants with the *TLR7* rs1634319 C allele had higher peak viral load (0.80 log₁₀ c/ml, 95% CI: 0.40, 1.20; corrected p=0.027).

For project 2, I found that all health care providers interviewed believed early, supported disclosure to children is important and cited concerns for the child's health and well-being as the central rationale. Providers viewed disclosure as a longitudinal process and advocated tailoring the approach to the individual child. Providers observed that preparation, support after disclosure, and a child's personality are more relevant predictors of the impact of disclosure on the child and family than the age when diagnosis is revealed. All stressed the need to incorporate caregiver preparation and empowerment and recognized that significant barriers to disclosure included caregiver fears about child reactions, including judgment of the parent.

Conclusions: I found that variations in *TLRs* influence HIV acquisition and progression in infants. These associations may inform novel vaccine and therapeutic strategies for pediatric HIV. My observations among health care providers revealed a wealth of clinical approaches that can be used in guidelines to improve pediatric HIV disclosure. Better understanding the mechanisms influencing infection and how to

care for HIV-infected children can help reduce the global burden of this disease.

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Introduction.

Since the HIV/AIDS epidemic began thirty years ago, HIV has caused more than 25 million AIDS-related deaths. Despite known prevention methods and a heightened research agenda, the HIV/AIDS epidemic remains devastating. There are currently an estimated 34 million people living with HIV [1]. The majority of those who are infected (69%) live in sub-Saharan Africa [1]. Increased HIV prevalence among women in Africa and incomplete uptake of prevention of mother-to-child transmission (PMTCT) services have resulted in a large number of HIV-infected children in Africa. Worldwide, more than 3.3 million children are infected with HIV and 330,000 more children become infected each year [2]. HIV-infected children in Africa account for >90% of the global population of children living with HIV [1]. The HIV/AIDS epidemic is reversing decades of progress made in increasing childhood survival rates in developing countries [3, 4].

In Kenya, an estimated that 180,000 children had HIV in 2011 and 13,000 infants acquired HIV [5]. According to the most recent Kenya Demographic Health Survey, childhood mortality rates remain high with 1 in 14 children dying before age five [6]. Estimates also suggest that 1/3 of infant deaths in Kenya can be attributed to HIV [7].

Not all children who are exposed to HIV will acquire HIV and there are differences in outcomes for children who become infected [8, 9]. Research is needed to better understand the mechanisms underlying observed differences in HIV outcomes in children. Acquisition of HIV, progression from HIV infection to AIDS, response to antiretroviral therapy (ART), and the specific opportunistic infections that infected individuals develop are influenced by demographic, behavioral, and genetic factors. Studies suggest that host genetic variations in innate immune system factors could play a critical role in HIV acquisition and disease progression. Better understanding these variations could elucidate new mechanisms underlying the heterogeneity in observed responses to HIV [10]. To date, host genetic studies have focused on components of the adaptive immune response and human leukocyte antigen (HLA), HIV co-receptor polymorphisms, and other genetic elements, have been shown to clearly alter susceptibility to HIV infection [11-19]. However, these genetic factors account for only 15-20% of the estimated genetic variability in HIV transmission and pathogenesis [10, 19]. Further studies are needed to

help characterize how genetic variations in other immune processes affect HIV susceptibility and disease progression.

Recent studies demonstrate that innate immune responses play a critical role in HIV acquisition and control [20, 21]. However, the role of the innate immune system in pediatric HIV infection remains largely unstudied. Toll-like receptors (TLRs) are critical proteins of the innate immune system that are responsible for viral and bacterial pathogen recognition and the initiation of adaptive defense mechanisms. Genetic variations in TLRs, especially those influencing mRNA expression, protein function, and regulatory binding site efficiency, may influence the ability of the innate immune system to recognize and respond to HIV. This may be especially true in infants where adaptive immune responses are still under development. Evaluating how genetic variations in *TLRs* alter the risk of HIV acquisition in infants and how these same polymorphisms affect progression in infants who become infected can help to address this gap in knowledge. Better understanding how *TLR* genetic variations influence HIV acquisition and disease progression can provide mechanistic information on how the innate immune system is involved in evading and controlling HIV infection and point towards the development of novel therapies and vaccines.

While it is important to figure out how to prevent infection, there are pressing, immediate issues facing the children currently infected with HIV. Due to expanded global funding for HIV treatment, children infected with HIV are receiving increased access to lifesaving antiretroviral drugs. As access continues to increase, more HIV-infected children will attain adolescence and disclosure of HIV diagnosis will become an increasingly important practice in pediatric HIV comprehensive care. Unfortunately, the majority of HIV-infected children are not aware of their HIV infection status. Primary caregivers and parents of infected children are often reluctant to inform children of their HIV diagnosis because of concerns regarding subsequent disclosure to others, perceptions of the child's inability to cope with results, and guilt regarding transmission. However, disclosure may significantly improve the health and well-being of the child. Disclosure of HIV status before sexual debut may also play an important role in preventing the spread of HIV infection among adolescents and young adults.

In addition to caregiver concerns providing a significant barrier to disclosure, lack of disclosure guidelines also pose challenges. There are currently limited guidelines on how, when, and what should be shared with HIV-infected children regarding their diagnosis. Given the lack of concrete guidelines for disclosure in resource-limited countries, current disclosure processes have been based on the findings of a few studies conducted in resource-rich countries, personal experiences of healthcare providers working within the country, and previous models for disclosure to pediatric cancer patients, also from resource-rich settings [22-27]. Despite these barriers, disclosure is still occurring in practice. It will be important to evaluate the current pediatric HIV disclosure processes taking place in resource-limited countries to gain information that can inform international and country-specific disclosure guidelines. This information can help with the development of disclosure guidelines that protect the child's emotional health while simultaneously allowing for the prevention of HIV transmission.

Chapter 1.

Toll-like Receptor (TLR) variants are associated with infant HIV-1 acquisition and peak plasma HIV-1 RNA level*

*This chapter is currently in press:

K.M. Beima-Sofie, A.W. Bigham, J.R. Lingappa, D. Wamalwa, R.D. Mackelprang, M.J. Bamshad, E. Maleche-Obimbo, B. Richardson and G.C. John-Stewart. Toll-like Receptor (TLR) variants are associated with infant HIV-1 acquisition and peak plasma HIV-1 RNA level. *AIDS, in Press*.

1.1 Abstract

Objective: We evaluated the association of single nucleotide polymorphisms (SNPs) in *TLRs* with infant HIV-1 acquisition and viral control.

Design: Infant HIV-1 outcomes were assessed in a Kenyan perinatal HIV-1 cohort.

Methods: Infants were genotyped for six candidate and 118 haplotype-tagging polymorphisms in *TLRs* 2, 3, 4, 7, 8, and 9, *MYD88* and *TIRAP*. Cox proportional hazards and linear regression were performed to assess associations with time to HIV-1 acquisition, time to infant mortality, and peak viral load (VL).

Results: Among 368 infants, 56 (15%) acquired HIV-1 by month 1 and 17 (4.6%) between 1 and 12 months. Infants with the *TLR9* 1635A (rs352140) variant were more likely to acquire HIV-1 by 1 month (HR=1.81, 95% confidence interval [CI] =1.05-3.14, p=0.033) and by 12 months (HR=1.62, CI=1.01-2.60, p=0.044) in dominant models adjusted for maternal plasma HIV-1 RNA level and genetic ancestry. Among 56 infants infected at ≤ 1 month of age, ≥ 1 copy of the *TLR9* 1635A allele was associated with a 0.58 \log_{10} c/ml lower peak VL (p=0.002). Female infants with ≥ 1 copy of the *TLR8* 1G (rs3764880) variant had a 0.78 \log_{10} c/ml higher peak VL (p=0.0009) and having ≥ 1 copy of the C allele for a haplotype

tagging *TLR7* variant (rs1634319) was associated with a 0.80 \log_{10} c/ml higher peak VL in female infants ($p=0.0003$).

Conclusions: In this African perinatal cohort, we found several *TLR* polymorphisms associated with HIV-1 acquisition and progression. Defining mechanisms for these *TLR* associations may inform HIV-1 prevention strategies that leverage innate responses.

1.2 Background

Recent studies demonstrate that innate immune responses play a critical role in HIV-1 acquisition and control [21, 28, 29]. This may be especially true in infants whose adaptive responses are still under development. Pattern Recognition Receptors (PRRs) are key effectors of the innate response that also bridge to adaptive immune response pathways. PRRs recognize evolutionarily conserved pathogen-associated molecular patterns (PAMPs) and PAMP recognition triggers activation of signal transduction pathways and downstream effector responses [30].

Toll-like receptors (TLRs) were the first mammalian PRRs discovered and 10 *TLR* genes have been identified in humans [31, 32]. Each TLR recognizes specific PAMPs characteristic of fungi, bacteria, viruses and/or parasites; TLRs 1, 2, and 4-6 preferentially recognize bacterial and fungal PAMPs while TLRs 3 and 7-9 preferentially recognize viral nucleic acids [21, 33]. Although TLR2 and TLR4 predominantly recognize bacterial motifs, they may also recognize viral components [31, 33]. TLR3 recognizes double-stranded RNA; TLRs 7 and 8 bind single-stranded RNA, and TLR9 recognizes unmethylated cytidine-phosphate-guanine (CpG) DNA motifs of bacteria and viruses [32].

All TLRs contain an extracellular leucine-rich repeat domain and an intracellular Toll/IL-1 Receptor homology (TIR) domain that binds to adaptor molecules involved in TLR-associated signaling cascades [30, 32, 34]. Biologic responses to TLR PAMP recognition are dependent on which TIR domain containing adaptor molecules are recruited and which signaling pathways are initiated. The TIR domain containing adapter-inducing interferon- β (TRIF)-dependent pathway induces the production of type I interferons and results in antiviral and immunoregulatory responses [30, 35] while the myeloid differentiation factor 88 (MyD88)-dependent pathway induces the production of pro-inflammatory cytokines and chemokines and induction of genes involved in antiviral response [34]. TIR domain containing adaptor protein (TIRAP), another TLR-adaptor molecule, functions mainly in TLR4 and TLR2 signaling either independently or in combination with MyD88 in the MyD88-dependent signaling pathway to upregulate NF- κ B and MAPKs [30, 34].

Because of their sentinel role in pathogen recognition and initiation of antiviral response, genetic variation in *TLR* and *TLR-associated* genes may influence HIV-1 acquisition and progression. Previous studies evaluating polymorphisms in *TLR2-TLR4* and *TLR7-TLR9* have shown that single nucleotide polymorphisms (SNPs) in *TLR* genes may contribute to differences in HIV-1 disease progression and acquisition [36-43]. Most notably, the *TLR9* 1635A/G variant has been associated with HIV-1 progression [38-40, 42]. However, the direction and strength of associations of this variant with HIV-1 progression differ between studies. The only study to date to evaluate associations between variants in *TLR9* and HIV-1 acquisition found a higher risk of HIV-1 acquisition in European children carrying a haplotype that included *TLR9* 1635A/G [43]. Studies in adults have also reported differences in HIV-1 disease progression or HIV-1 virus levels associated with SNPs in *TLR2* (597T/C), *TLR4* (896A/G and 1196C/T), *TLR7* (32A/T) and *TLR8* (1A/G) [36, 37, 39, 40]. Studies evaluating *TLR* variant associations with the presence of HIV-1 infection found that the *TLR7* 32A/T variant was detected more frequently in HIV-1 infected women compared to uninfected women [36] and the *TLR3* 1234C/T variant was significantly overrepresented in HIV-1-exposed seronegative (HESN) individuals when compared to healthy controls [41]. Other studies have shown correlations between levels of *TLR* mRNA, *TLR* protein expression, and *TLR* protein function with HIV-1 disease progression and acquisition in adult cohorts, further supporting the potential importance of *TLR* genetic variations for HIV-1 outcomes [37, 41, 44, 45].

The role of the innate immune system generally, and PRRs specifically, in pediatric HIV-1 infection remain largely unstudied. Furthermore, few HIV genetic studies have been conducted in African populations. We tested whether polymorphisms in *TLR* and *TLR-associated* genes are associated with altered risk of infant HIV-1 acquisition or disease progression in a perinatal African cohort.

1.3 Methods

Study Population and Sample

Our study used biological samples and phenotypic data collected from a cohort of mother-infant pairs recruited and followed between 1999 and 2005. As previously described, this cohort included 510 HIV-1-

infected pregnant women who were enrolled at ~32 weeks gestation and mother-infant pairs were followed up to 2 years postpartum [46-49]. Briefly, HIV-1 seropositive pregnant women received standard antenatal care and short course zidovudine (ZDV) from 34-36 weeks gestation through delivery for the prevention of mother-to-child transmission (MTCT) [50]. Women were counseled on safe infant-feeding practices and elected to either breastfeed or formula feed their infants. Infants were evaluated monthly during the first year of life. Infant HIV-1 status was determined by DNA PCR assays at 48 hours of birth and at 2 weeks and 1, 3, 6, 9, and 12 months of age. We limited the analysis to DNA samples linked to infants of known sex and for whom time to HIV-1 acquisition could be determined. All participants provided written informed consent for the primary research study and for use of samples and data in future research. The Kenyatta National Hospital Ethical Review Committee (ERC) and the University of Washington Institutional Review Board (IRB) specifically approved use of these biological samples and phenotypic data for this study.

Data Collection

Infant CD4 and CD8 percentages and lymphocyte counts were determined using a FACScan flow cytometer (Becton Dickinson, Franklin Lakes, NJ) at the University of Nairobi, Kenya. Viral load was measured at the Fred Hutchinson Cancer Research Center in Seattle Washington, USA. Specifically, plasma HIV-1 RNA levels were quantified using the Gen-Probe transcription-mediated amplification assay. Previous studies have demonstrated that this assay is appropriate for quantifying the HIV-1 A, C, and D subtypes that are prevalent in Kenya [51]. Infant peripheral blood mononuclear cells (PBMCs) were isolated from EDTA anti-coagulated blood using a Ficoll gradient (Lymphocyte Separation Medium; Organon, Teknika), washed in RPMI 1640 medium (Sigma-Aldrich), and counted using trypan blue staining under a hemocytometer. Cells were cryopreserved at the University of Nairobi and sent to the United States for DNA extraction.

Cryopreserved cells were thawed and washed in R-10 media (RPMI with 10% FCS) and 1% PBS (Sigma-Aldrich). DNA was extracted using the Genra Puregene Blood Kit (Qiagen, Valencia, CA) and eluted in 20, 25, and 50µl of hydration buffer depending on estimated cell pellet size. DNA was quantified using a

Spectra Max Gemina (Molecular Devices, Sunnyvale, CA) fluorimeter and the Quant-iT PicoGreen dsDNA Assay (Invitrogen, New York). Samples lacking 1µg of DNA (N=45) were whole genome amplified (WGA) using the REPLI-g Whole Genome Amplification Kit (Qiagen, Valencia, CA). Samples were genotyped using an Illumina Custom Oligo Pooled Assay (OPA) microarray platform (Illumina, Inc., San Diego, CA) designed for this study.

SNP Selection

We genotyped 124 SNPs in six *TLR* genes (TLRs 2, 3, 4, 7, 8 and 9) and two *TLR*-associated genes (*MYD88* and *TIRAP*). SNPs were selected using haplotype tagging and candidate SNP approaches. Haplotype tagging SNPs (TagSNPs) were selected using the LDSelect algorithm available through the University of Washington's genome variation server (<http://www.gvs.gs.washington.edu/GVS/>) using the r^2 threshold value of 0.8 and a minor allele frequency (MAF) cutoff value of 5% in the Yoruba (YRI) HapMap population [52, 53]. TagSNPs were augmented with six candidate SNPs, selected based on previously identified associations between variants in *TLRs* and HIV-1 outcomes [36-40] (Table 1.1). To assess population stratification, we genotyped 144 ancestry informative markers (AIMS) distinguishing Asian, European and African (West and East) ancestry.

Quality Control

We excluded samples in which there was a discrepancy between the reported and experimentally determined sex (n=5) or which had >10% missingness (n=24). SNPs were excluded from the analysis if they were monomorphic (n=2), had >10% missingness (n=8), or violated Hardy-Weinberg equilibrium ($p < 0.001$) (n=1). TagSNPs with a MAF <5% in our sample population were also excluded (n=18). Overall, 29 of 397 infants and 29 of 118 SNPs did not meet quality control criteria and were excluded from this analysis (Figure 1.1). In addition, five samples were genotyped in duplicate to estimate concordance rates in our genotyping platform. The genotype concordance rate was 100% for all successfully genotyped markers. Post-genotyping quality of WGA samples was evaluated and genotype frequencies for WGA and non-WGA samples were similar.

Data Analyses

Cox proportional hazards regression was performed to assess *TLR* polymorphism associations with time to HIV-1 acquisition by month 1 and month 12. Because viral load, CD4 count and ZDV use are collinear measures of maternal HIV-1 disease progression, genetic acquisition analyses were only adjusted for maternal viral load at 32 weeks gestation. Adjustment for population stratification was performed using the first principle component defined by EIGENSTRAT [54] analysis of genotyped AIMS.

Cox proportional hazards regression was performed to assess *TLR* polymorphism associations with time to infant mortality by 24 months in infants infected by month 1. Linear regression was performed to assess peak HIV-1 RNA levels in infected infants for infants infected by month 1 and month 12. All progression analyses were adjusted for genetic ancestry using the same method as the acquisition analysis.

All regression analyses were performed using Intercooled STATA 11.0 (College Station, USA). Sex-stratified analyses of the X chromosome genes, *TLR7* and *TLR8*, were conducted. For analysis with candidate *TLR* SNPs previously shown to be associated with HIV-1-specific outcomes, we did not adjust for multiple comparisons. All TagSNPs were adjusted for multiple comparisons using a Bonferroni correction to account for the 89 quality-controlled SNPs. All analyses were first performed assuming an additive model of inheritance. All candidate SNP analyses and TagSNPs showing uncorrected significant ($p < .05$) associations in additive models were rerun using dominant and recessive models of inheritance and the model best representing the association was selected.

1.4 Results

Cohort characteristics

Among 510 mother-infant pairs initially enrolled in a perinatal HIV-1 transmission cohort, 368 had samples available, passed quality control procedures, and were included in this analysis. Characteristics of these mother-infant pairs are provided in Table 1. Among the infants, 56 (15%) acquired HIV-1 by 1 month of age and 17 (4.6%) acquired HIV-1 between 1 and 12 months of age. Maternal CD4 counts at 32 weeks gestation were significantly lower in those who transmitted HIV-1 compared to those who did not

(406 vs. 483 cells/ μ l, $p = 0.011$). Plasma HIV-1 RNA levels were significantly higher among women who transmitted HIV-1 compared to those who did not, both at 32 weeks gestation (5.11 vs. 4.56 \log_{10} copies/ml, $p < 0.001$) and at delivery (4.66 vs. 3.93 \log_{10} copies/ml, $p < 0.001$). Most women (89%) reported use of zidovudine for the prevention of mother-to-child transmission. Women who transmitted HIV-1 before one month were significantly less likely to report zidovudine use than those who did not transmit (OR=0.338, 95% confidence interval [CI]: 0.159, 0.717; p -value=0.005). A similar association was noted for 12-month transmission (OR=0.43, 95% CI: 0.2, 0.89; $p=0.023$).

HIV-1 infected children experienced high rates of mortality with 45% of infants infected by 1 month of age dying before 12 months and 18% of infants infected between months 1 and 12 dying before reaching 12 months. Among the 56 infants HIV-1 infected at ≤ 1 month, the mean peak plasma HIV-1 RNA level was 6.8 \log_{10} copies/ml (\log_{10} c/mL) (Table 1.2).

HIV-1 Acquisition

The primary analysis endpoint was HIV-1 acquisition in infants. Infants with one or more copies of the candidate variant *TLR9* 1635A (rs352140) were more likely to acquire HIV-1 by 1 month (HR=1.81, 95% CI: 1.05, 3.14; $p=0.033$) and 12 months (HR=1.62, 95% CI: 1.01, 2.60; $p=0.044$) in dominant models adjusted for maternal plasma HIV-1 RNA levels and genetic ancestry (Figure 1.2). The *TLR9* 1635A allele association with time to HIV-1 acquisition remained significant at both time-points when evaluated using an additive model of inheritance and showed a trend for association at both acquisition time-points when assessed using a recessive model of inheritance. No other candidate or *TLR*, *TIRAP* or *MyD88* haplotype tagging SNPs were significantly associated with HIV-1 acquisition in analyses adjusted for multiple comparisons (Table 1.3).

Peak Viral Load

We found associations between *TLR9* 1635G/A (rs352140), *TLR8* 1A/G (rs3764880), and *TLR7* rs1634319 variants and peak plasma HIV-1 RNA levels in HIV-1 infected infants (Figure 1.3 and Table 1.4). The presence of one or more copies of the *TLR9* 1635A allele was associated with 0.58 \log_{10} c/ml

lower peak plasma HIV-1 RNA levels (95% CI: -0.95, -0.22; $p=0.002$) in infants infected by month 1 and with 0.49 \log_{10} c/ml lower peak plasma HIV-1 RNA levels (95% CI: -0.85, -0.12; $p=0.009$) in infants infected by month 12 using dominant models of inheritance. The association remained significant for both time points in additive models of inheritance and showed a trend for association with a recessive model of inheritance in infants infected by 1 month of age.

Female infants infected by 1 month of age and having one or more copies of the *TLR8* 1G allele (rs3764880) had a 0.78 \log_{10} c/ml higher peak viral load (95% CI: 0.35, 1.21; $p<0.001$); a similar association was seen in female infants infected by 12 months (0.65 \log_{10} c/ml higher peak viral load; 95% CI: 0.21, 1.10; $p=0.005$). The association remained significant at both infection time-points in additive models but not recessive models of inheritance. There was no significant association between peak plasma HIV-1 RNA levels and the *TLR8* 1A/G variant in male infants (Table 1.5).

We also found an association between a haplotype tagging intronic variant in *TLR7* (rs1634319) and peak plasma HIV-1 RNA levels. Female infants infected by 1 year of age with ≥ 1 copy of the *TLR7* rs1634319 C allele had a 0.80 \log_{10} c/ml higher peak viral load (95% CI: 0.40, 1.20; corrected $p=0.027$ assuming a dominant model of inheritance and controlling for genetic ancestry. A similar association was noted among female infants infected by ≤ 1 month (mean difference=0.75; 95% CI: 0.30, 1.19; corrected $p=0.17$), but this association was not significant after controlling for multiple comparisons. This variant showed no significant associations with peak plasma HIV-1 RNA levels in male infants. No other candidate or *TLR*, *TIRAP* or *MyD88* haplotype tagging SNPs were significantly associated with peak plasma HIV-1 RNA levels in analyses adjusted for multiple comparisons (Table 1.5).

Mortality

Because of potential survival bias, we evaluated time to death only in infants infected by 1 month of age. We found no significant associations with time to death by any *TLR*, *TIRAP* or *MyD88* haplotype tagging SNP included in this analysis after adjusting for multiple comparisons and found no significant associations with candidate SNPs; however we had limited statistical power to evaluate associations with

mortality. We noted a trend for association with mortality for two candidate SNPs (*TLR2* 1350T/C and *TLR7* 32A/T) (Table 1.6). In infants infected by 1 month of age, the *TLR2* 1350CC (rs3804100) genotype had a trend for association with increased risk of mortality (HR=6.36; 95% CI: 0.97, 51.24; p=0.082) using a recessive model of inheritance and controlling for genetic ancestry. However the minor allele frequency for this polymorphism was low (4.92%) and only 1 infected infant had this genotype. We also saw a trend for association between each additional copy of the *TLR7* 32T (rs179008) allele and time to mortality in female infants infected by month 1 (HR=2.84, 95% CI: 0.87, 9.23; p=0.082).

1.5 Discussion

Few studies have evaluated the role of *TLR* genetic variation in infant HIV-1 acquisition and progression [42, 43] and our study is the first to evaluate the influence of *TLR* polymorphisms in a perinatal African cohort. We found that *TLR9* 1635G/A was associated with both HIV-1 acquisition and peak HIV-1 viral load, and *TLR8* 1A/G and the haplotype tagging *TLR7* variant (rs1634319) were associated with peak plasma HIV-1 RNA level.

We found that *TLR9* 1635G/A was associated with a higher risk of HIV-1 acquisition early (<1 month of age), and by the end of the first year of life. This supports a trend observed in a previous study evaluating *TLR9* genetic variations in HIV-1 MTCT [43]. However, in contrast, this variant was also reported to be associated with protection from HIV-1 acquisition in an African heterosexual serodiscordant couples cohort suggesting that this variant may exert different effects in heterosexual and vertical transmission [55]. While this seeming discrepancy in findings could reflect population differences in LD, it is notable that the two cohorts that had the greatest likelihood for differences in population stratification with one African and the other European, shared a common biological mode of HIV-1 transmission and had similar *TLR9* association results. Conversely, the African heterosexual couples and our vertical transmission cohorts share a common population background (Eastern African) but were distinct in the biological mode of HIV-1 transmission. These findings could suggest that the source of the discrepancy is in the functional role that *TLR9* plays in heterosexual HIV-1 acquisition compared to mother-to-child HIV-1 acquisition.

We also found that the *TLR9* 1635A allele was associated with a decrease in peak VL. The protective effect we found associated with the 1635A allele is consistent with one previous study in adults of European ancestry [39] but contradicts the findings of two other HIV-1 progression studies in adults of European ancestry [38, 40] and one study in HIV-1 infected children of European ancestry [42]. Differences between these cohorts, including HAART use, population ancestry, and progression measures observed could have led to differences in *TLR* associations. Specifically, the timing during HIV disease course when these variants are being evaluated may have an impact in the observed phenotype for specific *TLR* variants. A differential effect of *TLR* variants on HIV-1 set point was noted in acute versus chronically infected African adults [55], and in a functional evaluation of TLR signaling in acute versus chronic infection [56]. In early HIV-1 infected infants, there is delayed containment or non-containment of HIV-1, making it difficult to evaluate set-point comparably to adults [57] and may contribute to the different effect we observed of *TLR9* 1635A on HIV-1 progression.

Our observation that *TLR9* 1635G/A was associated both with higher HIV-1 acquisition risk and lower peak viral load in HIV-1 infected infants may be explained by potentially distinct effects of TLR9 on HIV-1 acquisition versus progression. In the context of HIV-1 uninfected infants, this response could increase recruitment of activated CD4+ T cells that serve as targets for HIV-1 replication. We could speculate that the TLR9 variant was associated with recruitment of activated immune cells to infant mucosal environments where they serve as HIV-1 susceptible targets. However, TLR9 is primarily expressed on plasmacytoid dendritic cells and mediates interferon- α release and antigen presentation thereby linking the innate and adaptive immune responses [38]. Thus, TLR9-mediated pro-inflammatory cytokine release could also initiate anti-HIV mechanisms that limit viral replication in already infected infants and decrease peak HIV-1 RNA levels.

The specific function of the *TLR9* 1635G/A variant in HIV-1 disease remains unknown. The G to A variation is a synonymous change in exon 2, making it difficult to infer any specific functional outcome. However, an intronic *TLR9* 1174A/G variant associated with decreased TLR9 transcriptional activity [58] is found in high linkage disequilibrium (LD) ($r^2=0.98$) with 1635G/A in European but not African cohorts.

This could explain the observed differences in association of the *TLR9* 1635G/A variant and HIV-1 disease progression in these distinct ancestral populations. Thus, it is possible that, in our sampled African cohort, the *TLR9* 1635G/A variant is in high LD with a different currently unidentified causal SNP. Alternatively, *TLR9* 1625G/A could alter mRNA splicing or stability, or protein expression or function, which has been documented for other synonymous changes [59].

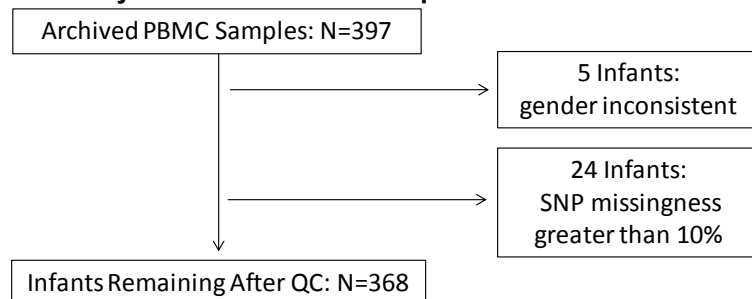
We found that *TLR8* 1A/G was associated with peak HIV-1 RNA level. We found that one or more copies of the *TLR8* 1G allele was associated with a 0.78 log₁₀ c/ml higher peak HIV-1 RNA level in female but not male infants. In contrast, *Oh et al.* found that the *TLR8* 1G variant was protective against rapid CD4+ T-cell depletion in males of European ancestry [37]. This difference in associations could be due to differences between adult and pediatric HIV-1 progression, gender differences, or differences in outcome measure assessed [60, 61]. Infants have much more rapid HIV-1 disease progression than adults which may be due to the relatively weaker adaptive immune response in infants [61]. We also identified a novel *TLR7* variant (rs1634319) associated with higher peak plasma HIV-1 RNA levels in female infected infants. *TLR7* viral RNA recognition can lead either towards immune activation and viral replication or an effective antiviral response [34]. The rs1634319 variant we identified is intronic but could influence *TLR7* expression or be in linkage disequilibrium with another causal variant.

While previous studies evaluating genetic variations in *TLR2* and *TLR4* have found positive associations with HIV-1 progression outcomes, our study did not confirm these associations or identify new associations with *TLR2* or *TLR4*, nor did our study confirm a previously observed association between a *TLR7* variant (32A/T) and HIV-1 progression in adult males [36]. Our study found no associations between genetic variations in *TIRAP* or *MYD88* and HIV-1 acquisition or progression. We may not have detected specific associations because of relatively limited statistical power, unique properties of vertical HIV-1 transmission that differ from sexual HIV-1 transmission, or differences in allele frequencies in our population compared to other previously published cohorts.

In summary, this study is the first to evaluate the association between *TLR* polymorphisms and HIV-related outcomes in a perinatal African cohort and confirms that variations in *TLRs* may contribute to pediatric HIV-1 outcomes. Further studies are needed to define mechanisms underlying these associations and the specific functions of *TLRs* during the course of HIV-1 disease.

Figure 1.1: Summary flow chart of quality control measures used for removing infants and SNPs from analysis

A: Quality Control for Infant Samples



B: Quality Control for SNPs

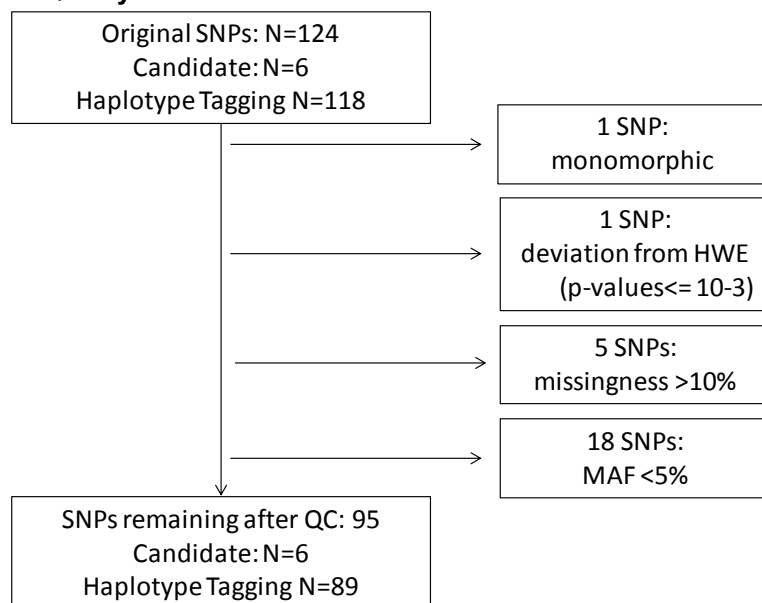


Table 1.1: Characteristics of candidate SNPs

Gene	Chromosome	SNP Reference Number	SNP	Type	Region	MAF	HWE*	Previous Study
TLR2	4	rs3804099	597 C/T**	coding - synonymous	Exon 3	0.30	0.44	Bochud [39]
	4	rs3804100	1350 T/C***	coding - synonymous	Exon 3	0.05	0.67	Bochud [39]
TLR4	9	rs4986791	1196 C/T****	coding - nonsynonymous	Exon 4	<0.01	N/A	Bochud [39]
TLR7	X	rs179008	32 A/T	coding - nonsynonymous	Exon 3	0.07	0.13	Oh [36]
TLR8	X	rs3764880	1 A/G	coding - nonsynonymous	Exon 1	0.31	0.78	Oh [37]
TLR9	3	rs352140	1635 G/A	coding - synonymous	Exon 2	0.29	0.02	Bochud [39], Soriano-Sabia [38], Pine [40]

*HWE – Hardy Weinberg Equilibrium; calculated only in uninfected infants

** Variant also referred to as 816 C/T

***Variant also referred to as 1569 T/C

****Variant also referred to as 1363 C/T

Table 1.2: Cohort Characteristics

Characteristic	Total Cohort		Positive by M1		Positive by M12	
	N	Median (IQR) or Number (%)	N	Median (IQR) or Number (%)	N	Median (IQR) or Number (%)
Maternal (Prenatal)						
Maternal CD4+ T Cell Count (32 weeks gestation)*	364	433 (306-619)	56	420 (237-597)	72	371 (237-558)
Maternal HIV-1 Plasma RNA Level (32 weeks gestation)**	355	4.72 (4.19-5.24)	54	5.20 (4.72-5.44)	71	5.19 (4.72-5.54)
Maternal (Delivery and Postpartum)						
Maternal CD4+ T Cell Count (1 month postpartum)*	323	540 (370-731)	49	469 (319-629)	62	466 (311-604)
Maternal HIV-1 Plasma RNA Level (time of delivery)**	293	4.12 (3.51-4.69)	45	4.52 (4.02-5.09)	61	4.65 (4.06-5.26)
ZDV During Pregnancy	361	322 (89)	54	42 (78)	70	57 (81)
Infant						
HIV Positive	368	73 (20)*	56	56 (100)	73	73 (100)
Female	368	178 (48)	56	29 (52)	73	33 (45)
Completed 12 Months of Follow-up	368	296 (80)	56	28 (50)	73	42 (58)
Completed 24 Months of Follow-up	368	41 (11)	56	20 (35)	73	28 (38)
Infant deaths (12 months)	368	48 (13)	56	25 (45)	73	28 (38)
Infant deaths (24 months)	368	62 (17)	56	33 (59)	73	39 (53)
Whole Genome Amplification	368	45 (12)	56	9 (16)	73	11 (15)
Peak HIV-1 plasma RNA level**	-	-	56	6.77 (6.43-7.41)	73	6.71 (6.41-7.40)

* CD4+ T cell counts measured in cells/ μ l

** HIV-1 plasma RNA levels measured in log₁₀ copies/ml

*** Infants positive by 12 months of age

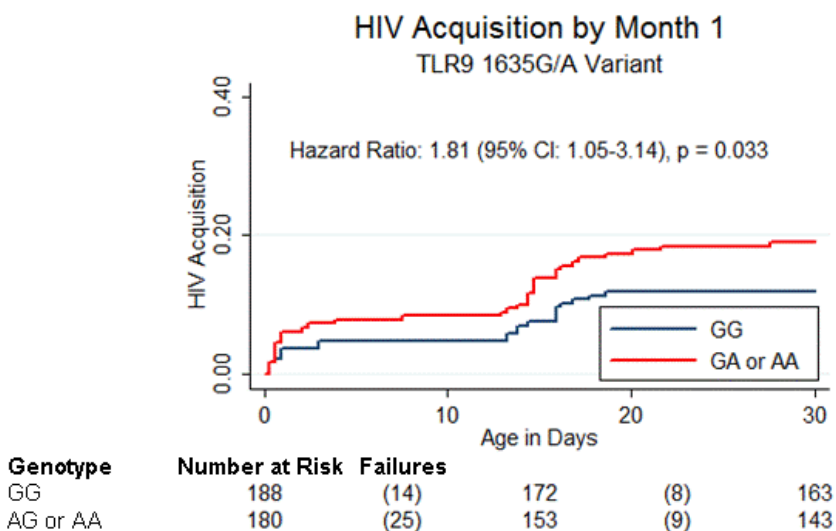
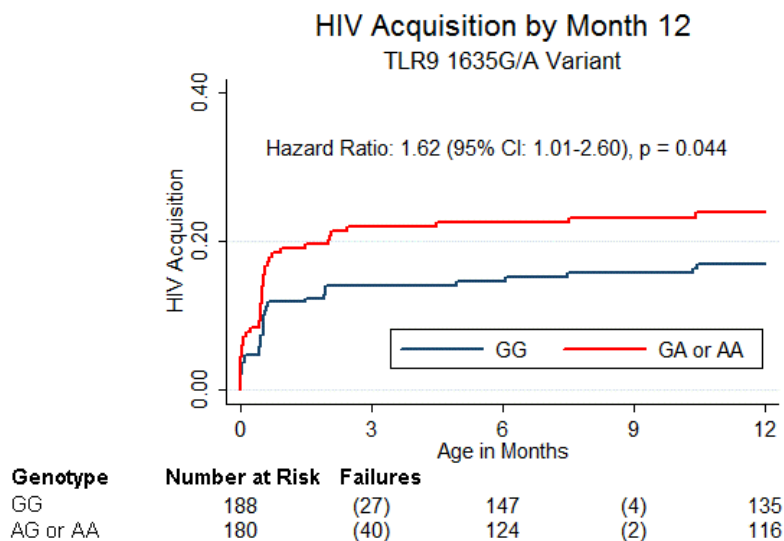
Figure 1.2: Time to HIV-1 Acquisition by TLR9 Genotype**Figure 1a:** HIV Acquisition in infants infected by 1 month of age**Figure 1b:** HIV Acquisition in Infants infected by 12 months of age

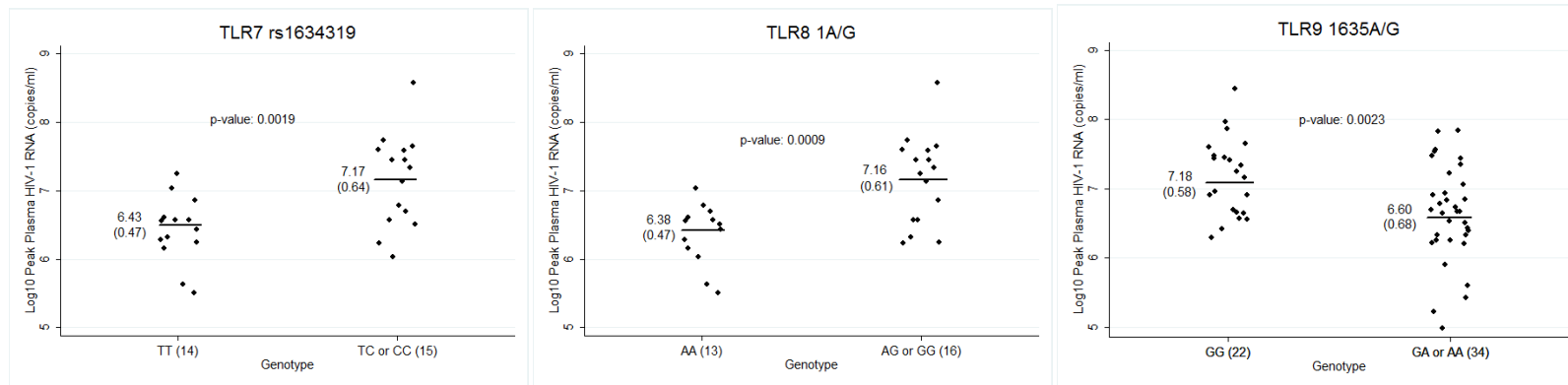
Table 1.3: Candidate SNP associations with time to HIV-1 Acquisition in Infants Infected with HIV-1 by 1 and 12 Months of Age

Gene	RS Number	SNP*	Time to HIV-1 acquisition in infants infected by 1 month of age				Time to HIV-1 acquisition in infants infected by 12 months of age			
			Model	Observations	HR (95% CI)	P-value	Model	Observations	HR (95% CI)	P-value
TLR2	rs3804099	597 C/T	Recessive	354	1.15 (0.46, 2.89)	0.7691	Additive	354	0.92 (0.64, 1.34)	0.6795
TLR2	rs3804100	1350 T/C	Recessive	354	3.37 (0.46, 24.26)	0.2293	Recessive	354	2.79 (0.39, 20.15)	0.3101
TLR4	rs4986791	1196 C/T	Additive	354	2.24 (0.54, 9.27)	0.2666	Additive	354	1.51 (0.37, 6.24)	0.5663
TLR7	rs179008 (M)	32 A/T	Male Only	183	1.51 (0.63, 3.63)	0.3527	Male Only	183	1.55 (0.65, 3.71)	0.3278
TLR7	rs179008 (F)	32 A/T	Dominant	171	1.29 (0.49, 3.40)	0.5996	Dominant	171	1.30 (0.49, 3.41)	0.5972
TLR8	rs3764880 (M)	1 A/G	Male Only	182	1.24 (0.64, 2.39)	0.5305	Male Only	182	1.25 (0.65, 2.42)	0.5039
TLR8	rs3764880 (F)	1 A/G	Dominant	171	1.07 (0.53, 2.15)	0.8573	Dominant	171	1.07 (0.53, 2.16)	0.8485
TLR9	rs352140	1635 A/G	Dominant	354	1.81 (1.05, 3.14)	0.0334	Dominant	354	1.62 (1.01, 2.60)	0.0443

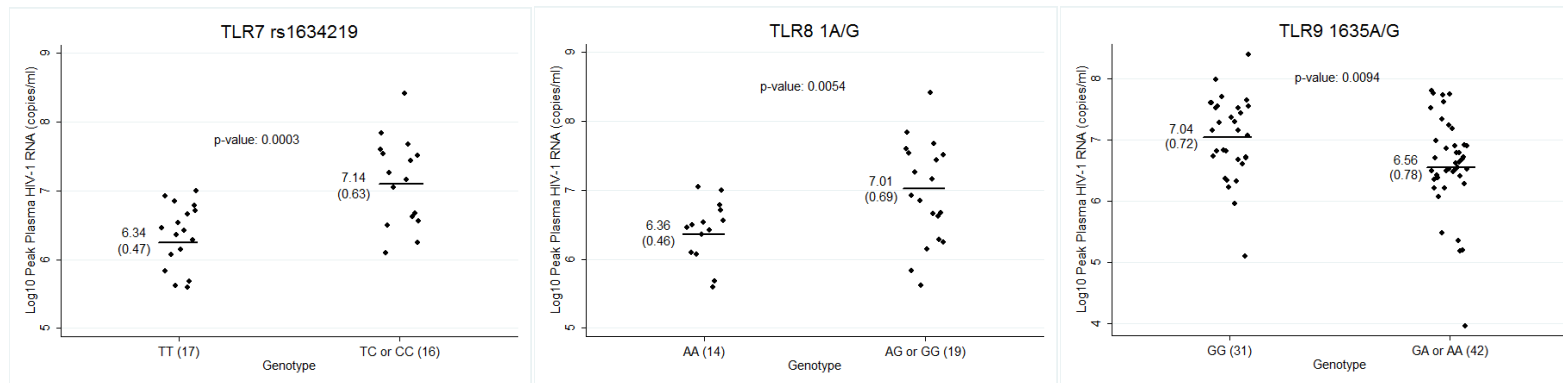
*M refers to males and F refers to females

Figure 1.3: SNP Variations Associated with Peak Plasma HIV-1 RNA Levels (\log_{10} copies/ml) in Infants Infected with HIV-1 by 1 and 12 Months of Age

Infants Infected by Month 1



Infants Infected by Month 12



NOTE: \log_{10} peak plasma HIV-1 RNA levels are displayed separately for infants who acquired HIV-1 by 1 month of age and all infants who acquired HIV-1 by 12 months of age. Each graph presents the mean and standard deviation of peak plasma HIV-1 RNA level stratified by genotype and the p-value corresponding to the mean difference. The number of individuals in each genotypic category is specified on the x-axis next to the genotype. Uncorrected p-values are displayed for the TagSNP *TLR7* rs1634319

Table 1.4: SNP Variations Associated with Peak Plasma HIV-1 RNA Levels in HIV-1 Infected Infants

SNP	Model	Genotype	N (%)	Median Peak Viral Load (IQR)	Mean (SD)	Mean Difference (95% CI)	p-value
Transmission by Month 1							
rs352140	Dominant	GG	22 (39)	7.12 (6.71-7.60)	7.18 (0.58)	-0.581 (-0.946, -0.217)	0.0023
		GA or AA	34 (61)	6.54 (6.28-7.00)	6.60 (0.68)		
rs3764880	Dominant - Female Only	AA	13 (45)	6.52 (6.28-6.60)	6.38 (0.47)	0.783 (0.351, 1.214)	0.0009
		AG or GG	16 (55)	7.27 (6.65-7.57)	7.16 (0.61)		
rs1634319	Dominant - Female Only	TT	14 (48)	6.50 (6.30-6.75)	6.43 (0.47)	0.746 (0.302, 1.189)	0.0019*
		TC or CC	15 (52)	7.40 (6.56-7.58)	7.17 (0.64)		
Transmission by Month 12							
rs352140	Dominant	GG	31	7.09 (6.57 - 7.60)	7.04 (0.72)	-0.485 (-0.847, -0.123)	0.0094
		GA or AA	42	6.54 (6.28 - 7.00)	6.56 (0.78)		
rs3764880	Dominant - Female Only	AA	14	6.49 (6.22-6.60)	6.36 (0.46)	0.652 (0.208, 1.095)	0.0054
		AG or GG	19	7.02 (6.46-7.56)	7.01 (0.69)		
rs1634319	Dominant - Female Only	TT	17	6.46 (6.06-6.71)	6.34 (0.47)	0.7999 (0.400, 1.200)	0.0003**
		TC or CC	16	7.27 (6.58-7.57)	7.14 (0.63)		

* Bonferroni corrected p-value = 0.17

** Bonferroni corrected p-value = 0.027

Table 1.5: Candidate SNP associations with Peak Plasma HIV-1 RNA Levels in Infants Infected with HIV-1 by 1 and 12 Months of Age

Gene	RS Number*	SNP	Peak viral load in infants infected by 1 month of age				Peak viral load in infants infected by 12 months of age			
			Model	Observations	Mean Difference (95% CI)	P-value	Model	Observations	Mean Difference (95% CI)	P-value
TLR2	rs3804099	597 C/T	Dominant	56	-0.165 (-0.560, 0.230)	0.4051	Dominant	73	-0.050 (-0.434, 0.335)	0.7981
TLR2	rs3804100	1350 T/C	Additive	56	-0.226 (-0.731, 0.279)	0.3732	Recessive	73	-0.557 (-2.155, 1.041)	0.4892
TLR4	rs4986791	1196 C/T	Additive	56	0.041 (-0.989, 1.070)	0.9368	Additive	73	0.069 (-1.073, 1.212)	0.9039
TLR7	rs179008 (M)	32 A/T	Male Only	27	0.307 (-0.670, 1.283)	0.5231	Male Only	40	0.384 (-0.416, 1.185)	0.3373
TLR7	rs179008 (F)	32 A/T	Additive	29	-0.180 (-0.918, 0.558)	0.6207	Additive	33	-0.095 (-0.821, 0.632)	0.7920
TLR8	rs3764880 (M)	1 A/G	Male Only	27	0.289 (-0.355, 0.932)	0.3636	Male Only	40	0.342 (-0.277, 0.961)	0.2702
TLR8	rs3764880 (F)	1 A/G	Dominant	29	0.783 (0.351, 1.215)	0.0009	Dominant	33	0.652 (0.208, 1.095)	0.0054
TLR9	rs352140	1635 A/G	Additive	56	-0.402 (-0.651, -0.153)	0.0021	Dominant	73	-0.485 (-0.847, -0.123)	0.0094

*M refers to males and F refers to females

Table 1.6: Candidate SNP associations with Mortality in Infants Infected with HIV-1 by 1 Month of Age

Gene	RS Number	SNP*	Model	Observations	HR (95% CI)	P-value
TLR2	rs3804099	597 C/T	Recessive	56	1.59 (0.56, 4.55)	0.3872
TLR2	rs3804100	1350 T/C	Recessive	56	6.36 (0.79, 51.24)	0.0820
TLR4	rs4986791	1196 C/T	Additive	56	-	-
TLR7	rs179008 (M)	32 A/T	Male Only	27	-	-
TLR7	rs179008 (F)	32 A/T	Additive	29	2.84 (0.87, 9.23)	0.0824
TLR8	rs3764880 (M)	1 A/G	Male Only	27	0.62 (0.18, 2.08)	0.4396
TLR8	rs3764880 (F)	1 A/G	Recessive	33	0.67 (0.15, 2.90)	0.5883
TLR9	rs352140	1635 A/G	Dominant	56	1.20 (0.59, 2.44)	0.6176

*M refers to males and F refers to females

Chapter 2.

Using Health Provider Insights to Enhance a Pediatric HIV Disclosure Model:

A Qualitative Study from Kenya

2.1 Abstract

As access to antiretroviral treatment has expanded, more children are receiving care. Yet many of these children have not been told their HIV status, particularly in high HIV prevalence regions. There is limited research on when, why, and how an HIV diagnosis is shared with a child. We evaluated disclosure in 5 large pediatric clinics in Kenya, caring for 287 to 1233 children. We interviewed healthcare providers with experience disclosing HIV status to children and evaluated their disclosure processes, experiences, attitudes and beliefs. Interview transcripts were coded and analyzed using modified grounded theory and thematic network analysis approaches. Themes were identified related to the disclosure processes, ethical and practical rationale for different approaches, and challenges or barriers to disclosure. Although working with limited guidelines and without disclosure protocols, providers described innovative approaches to discuss HIV with children, support systems, and empowerment procedures to assist caregivers in disclosure. Triggers for disclosure, timing and process of revealing HIV diagnosis to the child, and systems to support providers were characterized. Using insights from providers, we developed a model for disclosure that reflects the gradual individualized nature of the process including strategies used in practice, and balancing child and caregivers' values and concerns.

2.2 Background

Pediatric HIV disclosure is a rapidly evolving practice that has emerged as a challenge facing providers caring for HIV-infected children globally. Access to antiretroviral therapy (ART) has improved survival of HIV-infected children with an increasing number of HIV-infected children reaching adolescence. Consequently, more HIV-infected children are reaching ages at which the disclosure of HIV diagnosis is important. Disclosure may improve the physical and emotional health of the child and adherence to medications [62-65]. Yet despite these benefits, disclosure rates for children and adolescents in resource-limited countries remain low [63, 66-76].

Caregiver fears are the primary barrier to pediatric HIV disclosure [76], in part due to stigma surrounding HIV and because HIV often infects other family members. Disclosure also requires infected caregivers to come to terms with their own HIV status and to face possible feelings of guilt surrounding transmission to a child. Caregivers fear disclosure may lead to children blaming their parents for bringing HIV into the family; children being ostracized by peers or other family members; children inadvertently disclosing their family's illness which may result in stigma and rejection; or children being unable to cope with positive results [23, 77-86]. While studies have identified these concerns, few have identified mechanisms used to overcome these barriers to optimize disclosure.

To date, most research on pediatric HIV disclosure has focused on evaluating rates and correlates of disclosure and assessing outcomes associated with disclosure, but few studies have evaluated models of disclosure practice (22,27). Some studies offer recommendations for what an ideal disclosure process might encompass, however, these recommendations are not based on current practice or challenges and thus lack practical relevance to guide providers and caregivers through this sensitive process. To date, no studies have evaluated healthcare provider (hereafter referred to as "provider") decision-making processes for disclosure or how they justify and validate their decisions. Evaluation of decision-making rationale related to disclosure is necessary to inform the development of optimal disclosure processes.

The WHO recently published guidelines recommending that school-aged children should be informed of their diagnosis and younger children told partial information [87]. However, these guidelines fail to provide evidence-based best-practices for disclosure [87]. Kenyan guidelines also recommend disclosure to school-aged children but do not provide concrete techniques or approaches for implementing disclosure in practice [88, 89].

Despite limited guidelines and research, disclosure continues to occur in clinics. Pediatric providers (physicians, nurses, and counselors) have accrued considerable hands-on experience in practicing disclosure and provide a rich resource for disclosure protocols. Because providers experiment with different creative approaches to disclosure, they provide an untapped resource with a wealth of knowledge on how to optimize disclosure to HIV-infected children that can inform best practices guidelines. Thus, our study explored the processes, beliefs, attitudes and experiences of providers surrounding disclosure of HIV diagnosis using in-depth interviews with providers at multiple clinic sites throughout Kenya. We describe an experience-driven model for disclosure, identify rationale behind currently employed strategies, identify potential difficulties and gaps in practice, and describe creative techniques currently being used by providers that may inform the development of disclosure best practices.

2.3 Methods

Study Design and Population

This project used in-depth one-on-one interviews to collect qualitative data on the personal disclosure experiences of providers working with HIV-infected youth in Kenya. Providers were defined as any clinic staff member involved in the care of HIV-infected children including physicians, counselors, psychologists and nurses. Providers were recruited from 5 clinics throughout Kenya selected to represent a diverse array of settings including a large public tertiary referral and teaching hospital, a district level hospital, a private hospital, a faith-affiliated and supported clinic, and a small public clinic (Table 2.1).

Ethical Considerations

This study was reviewed and determined to be exempt from full review by the University of Washington IRB and went through full review and was approved by the Kenyatta National Hospital/University of Nairobi Ethical Review Committee. In addition, the protocol was reviewed and approved through private review processes for 3 study sites. All study participants provided oral and written informed consent.

Recruitment

We used purposive sampling to identify providers. Providers were recruited by clinic supervisors and lead pediatricians. Specifically, supervisors and lead pediatricians presented all providers at each clinic with an overview of the study and identified 3-5 clinicians with direct experience with disclosure and from different professional experiences at each clinic. The first author (KBS) or a trained interviewer met with these staff members, presented the research goals and procedures and re-affirmed willingness to participate.

Data Collection

Interviews were conducted in-person during June and July of 2012 and April of 2013. Interviews ranged between 30 and 90 minutes in length, were recorded using a digital audio recorder and later transcribed verbatim. Interviews were completed in English – excellent English proficiency is widespread among professional Kenyans. We used a flexible interview guide based on data from a previously published study by our group [76] and literature reviews. The interview guide was validated through informal focus group discussions with Kenyan providers in February 2011. Using this guide, we first asked providers a series of open-ended questions to explore their rationale and considerations in making decisions to disclose or withhold diagnosis including how they decide when and what to disclose and how they navigate interactions with caregivers. Second, we asked questions to determine the providers' perspectives on the process through which providers disclose, including what the general process entails, barriers to disclosure and successful strategies to facilitate disclosure. Additionally, we probed their opinions of how to improve the experience of disclosure. Memos were generated from the initial series of interviews to capture first impressions and details that may not have been captured in the recording.

Data Analysis

The goal of our analysis was to determine the experiences and processes, concerns, successes, beliefs and experiences of providers surrounding the disclosure of HIV status to children. Seventeen interviews were conducted by the first author and 4 interviews by a trained research assistant. Interview transcripts were coded using thematic qualitative analysis and modified grounded theory approaches [90, 91]. Using a modified version of the constant comparative approach [91, 92], an initial codebook was created by investigators KBS, MK, and BS based on a subset of transcripts. This codebook was revised and used to code an additional subset of transcripts used to further refine the codebook. All transcripts were read and coded independently by KBS and MK and a selected subset were reviewed by BS. Resulting codes and preliminary themes were discussed among all authors and revised. The chosen analytic framework focused on two basic components: (1) describing existing disclosure practices, including noted challenges, barriers, and successes (descriptive), and (2) eliciting provider's reasons and rationale for the timing and best approach for HIV disclosure with children and adolescents (normative). The descriptive perspective offers a window into current practice in a very high disease burden context and describes what is and is not working. The normative or evaluative perspective offers a deeper insight into the values implicit in developing best practices for pediatric HIV disclosure.

2.4 Results

Participant Characteristics

Our study included 21 providers, 3-5 from each study site. Participants had a range of clinical professions and included 7 clinical officers, 4 nurses, 4 nurse counselors, 2 psychologists, 1 clinic assistant and 1 peer educator. Participants ranged in age between 25-55 years and reported having between 1 and 17 years of experience working with HIV-infected children. Many participants mentioned only receiving brief disclosure training within trainings focused on other HIV issues. A few participants mentioned specific training in pediatric HIV disclosure, typically offered through the clinic. Participants' experience with disclosure ranged from limited to no personal experience to daily experience, including development of

clinic protocols and training others. Involvement in disclosure ranged from assessment and referral to personally facilitating the disclosure event defined as “when HIV is named to the child.”

Disclosure Model Development

Provider experiences were used to develop a model representing the overall disclosure process occurring in Kenyan clinics, informed by providers’ expert assessments of how to optimize that process and overcome barriers. This model consists of a disclosure assessment and decision-making process and the disclosure timeline (Figure 2.1). This model is grounded in the experiences of providers and based on their decision-making rationale. Four themes identified during provider interviews were used to inform the development of this practice-based model.

1 – Disclosure practices should balance child well-being with caregiver values

Providers felt that child well-being and caregiver values were both central components of the disclosure process. However, because these values often conflict, providers have the challenging task of balancing the benefits of disclosure for child well-being against caregiver values. Providers universally endorsed the importance of disclosure and identified many benefits that centered on the well-being of the child (Table 2.2). The main rationale for disclosing status, and doing so in a thoughtful way, was to support the health and psychological well-being of the child, which included improved participation in medical treatment. Although less emphasized, providers also recognized the importance of disclosure for preventing new infections. While providers recognized risks, they viewed these risks to be outweighed by the benefits.

“We had one of them who doesn’t talk up to date, you know. You ask questions and (get) just one word answers and that is it. He tells you what you want to hear. And he used to be playful, he used talk about anything. He just withdrew, after the disclosure....so it’s a risk. But I think the benefits outweigh the risks.”

- 006

Caregiver concerns and values were the most common reasons for delaying disclosure and the most commonly identified concerns and values included caregiver reluctance to tell others, fear of blame and guilt regarding transmission and fear of inability to answer the child's questions (Table 2.2). While caregivers often mean well in trying to protect their children from perceived harms in knowing their diagnosis, failure to disclose may actually cause more harm because children often suspect or know their HIV status and feel frightened and isolated. Recognition of the psychological harm that can occur from delayed or inadvertent disclosure motivated provider preferences for initiating a gradual disclosure process early in the child's life.

"You reach there [adolescence] and they just don't want to see you, they try to avoid and again mixing with others, like they isolate themselves, they have these suicidal minds, they want to kill themselves, and all that." - 003

"You can break them, you know, they cannot even comprehend whatever you are telling them. We have some of them who have just run away from home from the disclosure, so we have to assess all that." – 006

Almost all providers recognized the impact of disclosure on the caregiver and showed strong sympathy for and deference to caregivers.

"I saw disclosure happening, how a caregiver discloses to the child, and I know it is not easy. And from that day, when a parent tells me that they have disclosed, I say that I salute you for that. I am grateful that you were able to disclose it. It was not easy and we are here to support you, even after you have disclosed, we are here to support you." - 011

Providers did not view the caregiver as the barrier, but instead viewed the concerns and values of the caregiver as the barrier and felt that empowerment of the caregiver was the way to overcome this barrier.

Providers also viewed inclusion of the caregiver in disclosure as critical in ensuring child well-being and recognized that caregivers are wrestling with legitimate concerns.

"We should understand them because we should just imagine ourselves in their shoes. I think disclosure is not a very easy thing; it's very difficult, especially if a child reacts badly or if a child goes telling everyone. So we should just understand them and give them time and continue encouraging them..." – 017

2 - Disclosure should be gradual and evolving, not a one-time event

Providers identified a gradual disclosure timeline that involves 3 distinct phases; 1 - disclosure initiation, 2 - the disclosure event, and 3 - disclosure support and follow-up. This gradual disclosure approach allows for adequate preparation of the caregiver and child and development of a specific plan for disclosure that can be tailored to the individual child's developmental readiness.

"I can also talk to the parents and tell them how they can start initiating the child the process of disclosure, slowly by slowly, slowly by slowly, depending on the age.." - 005

"You have to move from known to unknown, because that's where we start from. You don't come in and start pouring out things to the child." - 010

Providers believe that well-delivered disclosure involves significant preparation, on-going follow-up, and feedback and support during and after the disclosure process. Initially, providers prepare the child using partial disclosure to help the child understand the basics of their condition and the importance of taking medication.

We tell them about the soldiers, do they know what the soldiers do? ...we have soldiers who protect us from these enemies which are diseases. "You remember when you were sick? It's because the enemy

was overcoming the soldiers but now you are taking your medicines and the soldiers are becoming stronger than the enemies. The enemy is being defeated, so you don't fall sick.” – 010

Once the process begins, the provider places emphasis on gearing the process for the child's understanding. During each visit, the provider, caregiver and child make incremental gains in knowledge letting the child lead the path to illness understanding. As the child gets older, providers switch to more age-appropriate terms like virus, immune system, and CD4 count.

When the child reaches an appropriate age, understanding, or some other pre-identified indicator, the disclosure event occurs (defined as the first time the word “HIV” is used to describe their illness to them). During the event, providers often describe using motivational counseling to allow the child to come to the realization of their diagnosis on their own as opposed to the direct communication: “you have HIV.”

Following disclosure, the child is monitored closely by the provider and caregiver. Follow-up post-disclosure involves assessment of child's health (medication adherence, emotional well-being), peer support (encouragement that HIV is not the end, there are others like me), and assistance building the provider-child and caregiver-child relationships.

Providers all believed that straying from a planned disclosure process can have negative consequences for the child and family. Providers believe that the child gaining knowledge of their HIV status is inevitable but used experiences to demonstrate that the way in which the child finds out influences impact of disclosure on the child. Providers recognized that some children have knowledge of their HIV status before being formally disclosed to and children who figured out they had HIV before being disclosed to rebelled, exhibited violent behavior, felt betrayed, and developed sometimes irreparable relationships with their caregivers.

“... until accidentally, the boy discovered that he is HIV positive and he became so violent like even ‘I don't want to take food in this house,....you are giving me this disease of the immoral people, this is a

disease for prostitutes and all that.’ And he got so angry, even when he comes to the clinic he is so bitter, he is kind of having a lot of bitter emotions within him and he is kind of exploding.” - 009

Common mechanisms of acquiring knowledge of HIV status before disclosure were through realizations that they were different from siblings or friends, media, reading signs or charts while in clinic, or overhearing private conversations about their health.

“[There] was one child, she learned her status through the media. It was very emotional for her. She went into a very bad state because she felt the mom lied to her. The mom wasn’t open to her and we had several sessions with her and finally she is fine she is ok, she doesn’t feel alone.” - 011

Providers also identified that abrupt or disruptive disclosure, without preparation, could negatively impact the child’s health.

“We had one case where he is an orphan but the aunt was the guardian. He ended up being so promiscuous. He is 13 (years) but he has this huge body, you know. He rapes or defiles everyone. The other time the police came. They wanted a report from us. That is so terrible because the aunt, who really didn’t care disclosed. The aunt used to tell him ‘if you don’t take your drugs, you will die, you know you have HIV, you have to take your drugs.’ And that is disclosure. So he ended up rebelling.” - 006

“We have one experience, [a] boy, he was 14 years..., and we were going through the disclosure process but somehow on the way, disclosure was done in a wrong way. I think the sister was calling out his name and they were playing outside with the boys and she shouted, ‘Come and take your medicine, do you know these are for HIV. And if you don’t take you will die.’ And from that day, that boy changed completely. He doesn’t want any relationship with the sister. In fact there was a time he was saying, ‘Me, my sister died.’ When he comes to the clinic, he doesn’t want to talk to us. He doesn’t want to see us. He used to throw insults at us in the session and just leave, since then the behavior has never changed. He

comes, looks at you, you call his name, he says 'I am not (Mentioning his name).' He doesn't even want to be called that because the way disclosure was done was really, really bad." – 010

Overall, providers believed that regardless of the mechanism revealing the diagnosis, children who acquired knowledge of their status through unplanned processes were at greater risk of having adverse reactions to learning their diagnosis. Based on this, all providers advocated that disclosure be done early, before the child finds out their status inadvertently, and through a supportive environment.

3 - Pre-identified indicators let the provider and others know that the disclosure timeline should be initiated or advanced

Before initiating gradual disclosure, providers perform a detailed assessment of child and caregiver readiness for disclosure to determine the pathway through which the child enters the disclosure timeline. Through the assessment and decision process (Figure 2.1), the provider evaluates whether the child is ready for disclosure by identifying whether they have reached pre-identified triggers (Table 2.3). Triggers clustered into 5 distinct categories including: 1) child's age, 2) child's level of understanding, 3) child's personality, 4) upcoming child transitions in school, and 5) the child's social situation. While providers placed significant weight on age and maturity, no single trigger typically determined the timing when the child was determined ready to initiate gradual disclosure. Instead, providers described considering a constellation of these triggers, the combination of which is unique to each child. Thus, even if guidelines in a particular clinic specified "Begin disclosure between ages 7-8," providers were actually making decisions by balancing several considerations. Criteria varied by provider, and some placed more weight on certain triggers than others to ensure an optimal approach for each child.

"Initially the age used to be at 7 and 8 years but now you realize some of the kids start being inquisitive as early as 4 or 5 years, so it becomes tricky.... So it depends on the kind of child you are dealing with. Because some of them, they are not even inquisitive. So you can wait up to 7 or 8 years. But for the ones who start asking questions as early as 4 years, then you have to start telling some a few things. So it varies from child to child." – 006

Providers recognized that certain triggers may prompt immediate disclosure. These included the child directly asking the parent or recognition that the child may be starting to figure out their diagnosis independently.

Because these providers all viewed disclosure as a process that balances needs and concerns of the child and the caregiver, they all described simultaneously assessing child and caregiver readiness. The provider assesses the caregiver's readiness by evaluating their fears and concerns, psychological state, and knowledge of HIV. If the caregiver is not ready, the provider will postpone disclosure, work to empower the caregiver to make disclosure possible, and return to the assessment phase. Providers also reported that assessment should include the identification of accessible support resources for both the child and the caregiver

Assessment results lead the provider to choose one of three different pathways through which the child enters the disclosure process including the ideal pathway where the child initiates the gradual disclosure process, a more high risk pathway where the child immediately learns their specific diagnosis, or the potentially harmful pathway where the provider determines that the child already knows their diagnosis and steps in to provide support and follow-up for the child.

4 - Optimal disclosure ought to involve the caregiver and the provider; both play integral but specialized roles

Providers identified distinct, although sometimes overlapping or conflicting, roles for themselves and caregivers during disclosure. Based on their overarching belief that caregiver involvement is necessary to ensure the well-being of the child, all providers noted that disclosure will not occur in the absence of caregiver consent.

"If the caregiver is nervous and resistant, we just try to give them continuous counseling...because if a parent really does not want you tell the child his status, you will not say...." - 002

Even in cases where it is obvious the child already knows their status, providers waited for approval from caregivers.

“We have a child who is around 15 (years), but the parents still don’t disclose. But from what we see, we see that she has the information because she’s been sitting around with other adolescents who come in, but it is just that the parent has not actually sat this child down and discussed this with them, and we are waiting for the parent to give us consent, so sometimes there are those challenges.– 015

Although giving ultimate control to the caregiver, almost all providers thought it important to persuade caregivers to disclose sooner rather than later.

Providers viewed themselves as having a dual role in the disclosure process; to provide medical information and assessment and to provide emotional support. Lack of caregiver readiness indicated to providers that they needed to postpone disclosure and shift to educating, empowering, and persuading caregivers on the importance of HIV disclosure. Providers identified strategies through which they empower the caregiver including: 1) coaching on how to bring up disclosure and words and phrases to use, 2) preparing them for what to expect and how to identify concerning behaviors in the child, 3) stressing the importance of gradual disclosure, 4) encouraging caregivers to build on what the child already knows and answer the child’s questions truthfully with age-appropriate information and 5) ensuring that caregivers had correct information about HIV to share with the child. Providers also stressed the importance of making sure caregivers were psychologically prepared to support the child. Providers described strategies of sharing previous success stories to give caregivers hope and guiding them towards support groups to access support.

Providers believed that the caregiver’s role includes assessment of the child, assisting in disclosure initiation, performing the disclosure event (naming HIV), and provide support to the child after disclosure. All providers strongly believed the naming of HIV should come from the caregiver. In most cases,

caregivers know the child best, have a trusting relationship with the child, and are the ones who will be caring for the child and in the best position to watch for troubled responses after disclosure.

"The child does not have a lot of connection with the provider because we only meet once in either a month or twice or once in two months. But the caregiver is there with the child every day and the child has developed trust on the caregiver or the guardian and so it is better for the guardian to speak it to the child, the child trusts the guardian more than the clinician." - 001

While providers were generally clear in their rationale for deferring to caregivers on the disclosure event, it was unclear whether some providers might also defer to parents because the act of disclosure is very emotionally challenging.

"I think the caregiver or the parent is still the best person to give this information, because any other question the kid would ask about the family, he or she would be the right person to answer. We are just there to give the medical information that we have." - 015

Providers identified that caregivers often disagreed with their opinion that the naming of HIV should come from the caregiver. Providers thought that caregivers asked them to name HIV because they thought the providers were experts and found the naming of HIV emotionally challenging.

"They kind of want...to refer them to you because they feel that you are better, yet it is something that almost I am learning it as I do it each and every time." - 001

"We have always encouraged the parents to do disclosure because they are the key role people over this child. But they fear the blame so they tell you, just tell the child because you are the expert." – 014

Over half of the providers believed that they could be involved in the disclosure event when the caregiver asked for assistance, felt the child needed professional advice, or did not have a good supportive

relationship with the child. However, many providers expressed discomfort in taking on this role. They felt as though their relationship with the child did not extend to this role, that they had limited experience, and feared the blame or potential legal action that might follow a bad disclosure.

“They do ask, but I tell them it’s about them and the children. So I always tell them that it’s their responsibility and I give the reason as to why it’s theirs and not mine.” - 010

Best Practices for Disclosure

Providers identified logistical challenges in implementing optimal disclosure processes. Providers also identified creative techniques for disclosure that are working well in practice (Table 2.4). These insights can help inform the development of more specific trainings and concrete guidance for disclosure practices.

Clinic logistics and the child’s social context present major challenges for providers

Providers identified clinic logistics and the child’s social context as challenges affecting disclosure (Table 2.4). Providers all believed that it was important to create rapport with the child before discussing disclosure-related topics. However, the limited amount of time available to spend with the child and inconsistency in continuity of care created challenges in creating rapport. Providers also identified time constraints, influenced by large child to staff ratios (Table 2.1), as a barrier to creating this relationship and individualizing disclosure. Within the child’s social context, one of the most challenging issues providers experienced were situations where the provider knows that the child knows their status, but the caregiver remains unwilling to officially disclose to the child.

“They know it maybe from books, from magazines, from TV, from internet and you will find that they will keep quiet. They will not even talk to the parent about it, yes. But when they come to the session, it’s when they will tell us, “yes I saw my medications, I googled somewhere and saw...” And so the parent is seated there and they are like, my child does not know, but they know. So we call in the parent and explain this is what is happening, your child has already known their status, through this.” - 009

Providers also noted challenges related to the family social structure. For example, when fathers, acting as the head of the household, block disclosure but refuse to come to the clinic or be involved in the child's care. Some providers observed that lack of paternal involvement led to rebellious behavior and depression in children during and after disclosure.

Providers also recognized many challenges specific to disclosure in orphaned children. Uninfected caregivers who are not biological parents may have trouble disclosing because they have less information about HIV and lack personal knowledge of the disease. Some have additional hurdles to overcome before disclosure can happen such as how talk about death of parents while others simply do not know the child as well as a parent would, or do not have the same level of trust.

"He was an orphan, he is an orphan actually. And the aunties didn't actually want to disclose. So when I talked to the aunty and I told her you need to disclose to the child, the aunty said no, no, no, no, no. I cannot disclose because even the time the mother died, the boy was not told that his mother died, so up to date the boy does not know that his mother died, he only knows that his mother travelled somewhere far, yeah. So the aunty actually did not want at all, she said it will traumatize the boy, we cannot disclose."

- 002

Providers also observed that the messages used in public health prevention campaigns often conflicted with the positive messages required for disclosure. For example, many children in Kenya are told via public health messaging that HIV is "a killer disease" or the consequence of promiscuous sex. When children learn their diagnosis it can be stressful and devastating for a child who has been exposed to fear-based messaging.

"You want to correct the information they have , some of the information that is given to these children is that HIV is a very dangerous disease, it's a killer disease, once you get that, it's over with your life, so you want to correct that information to let them know that even if they maybe having HIV, there is life after

HIV, there is treatment, there is something that can be done to support the HIV positive people and they can lead normal life just like other children, other young adults and even achieve their dreams that they have in life. Because if someone discloses the status of their children or their youth, when they have a wrong mentality that HIV is a killer disease now, it's kind of...you are putting this child into depression,. 'I have HIV, it's a killer disease, now what happens to me.' So you want to correct that information". – 009

In addition, many children remain unaware of mother-to-child transmission (MTCT) and about how they could acquire this sexually transmitted disease. Behavior-change public health messaging can also affect how children view their parents' behavior when they learn their diagnosis. This association of HIV with promiscuity and unfaithfulness can make caregivers reluctant to disclose and fear how their child will think of them after learning their diagnosis. A child may blame their parent for being promiscuous or even immoral, and providers reported that biological parents often have significant anxiety about their child's reaction about transmission.

"Most of the healthcare workers, the teachers, they emphasize so much on the sexual part of it so that by the time they are coming, the time you are disclosing, they are already thinking, 'you are so promiscuous mom', you know. They already have an opinion. So that by the time you are telling them, they don't take it so lightly. Some of them, by the way, end up running away from home, others just withdraw." – 006

Targeted trainings and national networking can bridge gaps between current guidelines and provider-identified needs

Overall, providers felt that there was a lack in guidance on how to implement disclosure processes and variation and confusion about best practices. Providers felt that current practices being implemented at their clinics were based on their personal experiences rather than guidelines and trainings.

"I think sessions are better off if there is a structured way , like you see, I am just saying it from the way I have been handling the kids. I have experience in counseling, I have gone to some training in counseling

which involved a small bit on child counseling, so not like really I'd say on a professional basis I would do this and this but on an experience basis I'll say that I would do this and this, you know." - 004

Providers also felt that disclosure practices would benefit from national standardized operating procedures and opportunities to network and share experiences surrounding disclosure with others. Providers mentioned that separate training modules on pediatric HIV disclosure were lacking and almost all recognized the importance of receiving training in disclosure to help them feel more confident and capable of tackling this challenging topic.

"Before I was trained on disclosure, it was a terrible experience.....But after learning how to disclose, it has been not smooth, I cannot say it has been smooth still, but because I have the skills, I am aware of what to do at what point." - 014

Providers also recognized that not all children communicate through verbal dialogue and that training on how to incorporate alternative forms of communication, such as play therapy, would enhance the disclosure process.

Creative techniques currently used in practice can facilitate the development of best practices

In light of limited guidelines, many providers were proactive and came up with their own best practices. Providers identified a range of creative techniques they employ to optimize disclosure in practice. Many of these techniques focused on the need to provide comprehensive support for children, caregivers, and providers during disclosure. All providers in the cohort believed that disclosure should occur in a supportive environment and noted that joint parental disclosure and embracing infection as a family often leads to improved outcomes in the child. Disclosure allows HIV be a shared burden and reduces the stress associated with keeping that secret. One provider viewed disclosure as a way to enhance the entire family relationship.

“Disclosure is important because once disclosure is done, the family will be knitted together. They will now not look at this child differently, but now they will support the child.” – 014

Providers conveyed the importance of supporting caregivers to help them cope with their own challenges and noted that caregivers may get more out of peer support groups than in meetings with providers.

“There are issues these mothers are not telling you. And the moment they come in a support group, they will even learn from those others how they managed to deal with those issues and this one will also learn.” - 013

Providers also noted that comprehensive support following disclosure, regardless of the child’s clinical outcomes, and access to peer support were important determinants of the impact of disclosure on the child.

“In fact when they come into a group with other children, everything goes well, because at that point they know, “Oh, so am not alone. There are so many other children who are like me.” Their lives completely change and they are ok and happy.” - 010

Support for providers was also noted as beneficial since providers experience stresses associated with balancing caregiver reluctance with their own feelings of urgency and often find disclosure to be emotionally difficult and draining.

“This is an emotional kind of a job, so we need to be helped emotionally, so that we can be able to help other people emotionally.” - 011

2.5 Discussion

Our study found that despite few practice guidelines, providers in different clinics who are largely left on their own to figure out what specific approaches work best, have arrived at quite similar strategies and

views about optimal disclosure processes. We summarized the experiences providers described by generating a practice-based optimal disclosure model. Providers universally believed disclosure was important for ensuring the children's well-being and medication adherence and observed challenges with deferred disclosure. Providers also emphasized the value of having caregivers involved in the disclosure process and made significant effort to empower caregivers to disclose. We found that providers had defined roles for themselves and caregivers in disclosure and used a combination of indicators to make disclosure decisions. We also identified logistical challenges, gaps and needs, and techniques facilitating success that can be used to develop disclosure best practices.

Providers emphasized the need to consider the child within the family and social context and to tailor disclosure to each child. Despite mentioning time constraints as a logistical challenge of busy HIV clinics, providers put significant time and thought into helping children through the disclosure process. Providers recognized that disclosure should be geared towards the child with appropriate preparation, through building relationships and knowledge, before naming HIV to the child. Providers also emphasized the need to help caregivers come to terms with their own HIV status first, an issue that is underappreciated in current guidelines.

Our study found that child, and particularly adolescent, autonomy in dealing with the HIV diagnosis is often defined by the child's ability to transcend obstacles within the school environment. Attending school required ownership of the responsibility for caring for oneself. Providers and caregivers will need to focus on school as a critical point because disclosure is tied to this developmentally meaningful "rite of passage" in the child's understanding and acceptance of their illness. This important transition will need to be addressed in future guidelines.

We identified an important link between medication and perception of illness that influences both the disclosure process and the child's acceptance of diagnosis. Providers identified the child's questioning or refusal taking medication when he/she does not feel sick to be an important trigger for disclosure. Medication adherence also emerged as an important symbol of the child's acceptance of diagnosis, as

well as a potential symbol of illness ownership for those children who feel substantially empowered and confident. Overall, we found that providers and caregivers see medication adherence as central to the child maintaining health, which is what they communicate to the child during disclosure.

Providers also identified a need to address the tension presented in public health messaging versus the positive HIV messaging needed to support disclosure to children. At a programmatic level, and in the local culture, fear has been used as an HIV prevention tool. However, at an individual level, providers need to counter programmatic prevention messages to ensure psychological well-being of the child. With a growing HIV-infected adolescent population, it will be important to consider whether the public health impact of preventive messaging using fear tactics is worth the cost in terms of the negative impact this messaging can have on infected children when they learn their status.

An overriding theme across all providers was the importance of social support for children before, during, and after the disclosure process. Providers in our study identified that a positive home situation can make the difference in whether disclosure happens at all, and whether it's done well or poorly. Our study also revealed two key social factors that impact supportive disclosure and continuity of the disclosure process. Fathers were often perceived to represent a barrier to disclosure for the child's mother, suggesting a need for targeting education toward men on the importance of disclosing status to a child. Providers also reported that orphaned children and teens living without biological parents are at greater risk of "falling through the cracks" when it comes to timely, supported disclosure. Given the strong emphasis on deferring to primary caregivers, if guardians of OVCs are unable or unwilling to take responsibility for supportive disclosure, the disclosure process will be compromised. The burden may need to be shifted to providers to be more proactive in disclosure than they might be with parents or more involved guardians. This suggests a need for targeted programming to address this population of especially vulnerable children.

Our study was conducted at a diverse group of sites. We recognize that these sites cannot encompass all disclosure experiences and will not necessarily be generalizable in some settings. However, we believe

that the experiences providers shared with us can begin to fill a critical gap in knowledge around how and why providers make disclosure decisions, as well as inform evidence-based practice guidelines that are more responsive to the real challenges faced by providers every day.

Currently available disclosure guidelines do not accurately address or reflect the challenges providers are encountering in practice. Our study was designed to investigate the practical, social, and ethical challenges and attitudes surrounding disclosure from the perspective of health providers in Kenya. Generating best practices for disclosure should harness provider-identified creative solutions and address challenges and gaps in current practices. This data should be used to further optimize the disclosure model we outlined being used by providers in practice. In addition, future studies should seek to incorporate the experiences of HIV-infected children who have been through the disclosure process and caregivers of HIV-infected children to further inform disclosure decisions, processes and best practices. Understanding disclosure comprehensively through multiple stakeholder perspectives can inform the development of concrete disclosure practices that consider practical barriers

Table 2.1: Clinic, Healthcare Provider and Children Characteristics

Characteristic	Clinic 1	Clinic 2	Clinic 3	Clinic 4	Clinic 5
HEALTH FACILITY					
Type of clinic	Public	Public	Public	Faith-based	Private
Health facility level (2 – 6)*	3	4	6	N/A	N/A
CHILDREN IN HIV PROGRAM					
Total children	468	287	965	1233	1054
Children age 8 – 17	247	145	475	589	356
Aware of status	50%	40%	40%	55%	70%
Clinic Burden (# Children/# Providers)	25	17	97	123	53
HEALTHCARE PROVIDERS					
Providers	19	17	10	10	20
# Interviewed	5	3	4	5	4

*Kenya Ministry of Health classification of health facility level: 2 – dispensary, 3 – health centre, 4 – sub-district hospital, 5 – district or provincial hospital, 6 – tertiary referral hospital

Figure 2.1: Pediatric HIV Disclosure Processes in Practice: A provider-identified process-oriented model

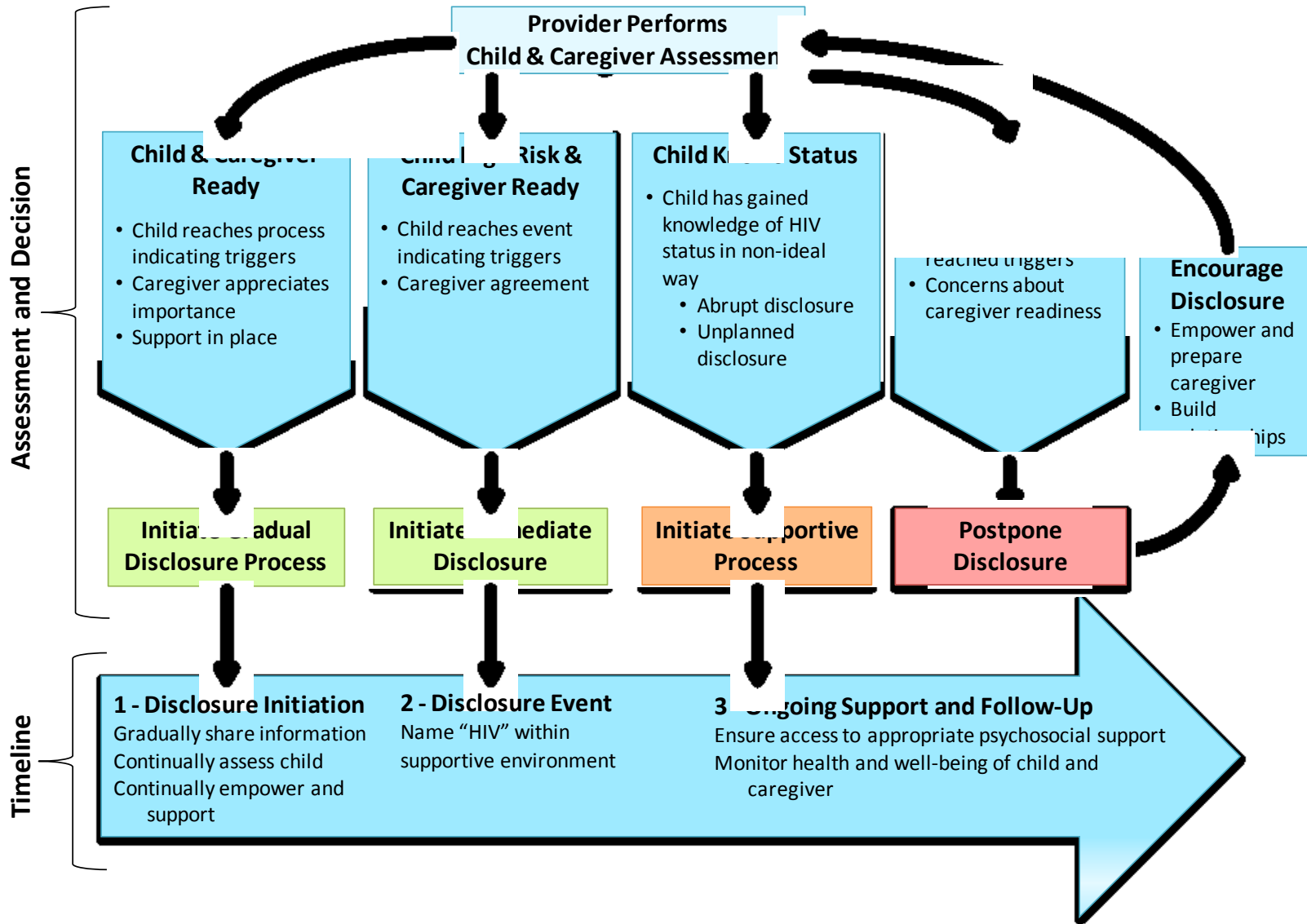


Table 2.2: Provider Identified Benefits and Barriers to Disclosure

Reason	Representative Quote
Benefits and Positive Impact of Disclosure	
Overcome Stigma/Discrimination	"When there is no disclosure, the child will be stigmatized in school. But once we disclose early, the child will have self-defense. They can even be able to share this with their friends, I am living positively and I am HIV positive and that is the time the child will be able to take care of herself. Timing of drugs will be no problem because she has developed defense, self-defense." – 012
Right to Know	"I think first it is a human right. Anybody wants to know what is happening to them, anybody, even a child. They want to know what is happening to them, so it's important just to disclose, so that I can know this is what is happening to me, so that if I am doing anything, I'm making informed choices from what I know." - 015
Independence/Autonomy	(From child's perspective) "At the end of the day, I'll be able know who to share information with everyday, to make informed choices for my future, how I want it to be." -015
Preserves Trust	"They end up being close and trusting their parent.....So for us, it's a benefit. We haven't disclosed, they haven't heard it from school, they haven't heard it from the media, they heard it from you (the parent), they end up being friends with the parents." – 006
Creates Self-Esteem	"Like this particular girl, she comes around for her drugs, she takes herself back to school and she is happy about it and that encourages me. Whenever I see her, I smile and she smiles back because she knows where I have taken her from. This is a girl who was brought here by the guardian, both parents had died and from there her elder sister just carried her, moved with her, and now she is ready to tell the world that yes, I knew my status when I was young and here I am." - 013
Ability to Receive Peer Support	"They get to be involved in support groups that help them to share their experiences with their peers and with that now they get to know that am not alone, I have other peoples around me. I have support, I have other friends who are my age, who are going through the same thing and I can receive support from them." -009
Protect Themselves	"As long as they know their status, they will know how to protect themselves. It would be devastating for a child who doesn't know their status and they have sex with their boyfriend or girlfriend, they get infected, and then they come to know later they are positive, they start now feeling guilty for having infected their partner, so it is important in that perspective." – 011
Medication Adherence	"It is very important because it helps them in adhering well to their medication. Once they know the problem they have, they will actually try and adhere to medications, because they know, if I don't take my meds, I'll be sick, yeah. So it is important for adherence, majorly for adherence." -002
Physical Health	"Another one again, to improve good health of the child, improve the CD4 count and reduce the viral load of the child also." – 005

Psychological Health	"Those who are disclosed to later, even they have suicidal thinking, maybe why, maybe he is the youngest in the family. They have that feeling, why me alone, and they will even ask questions when we are here, "I want to confirm my status today and today," or even ask the mother, "was it a must for me to breastfeed," so they will be adolescent stage. You know that fight is challenging and it is traumatizing to the mother because of such questions. But for early, they will grow knowing that and they will learn more things about HIV. If so and so have lived, even me I'll live." -012
Prevent Infecting Others	"The disclosure is also good because it will help the child to care about themselves, they will take care of others and they will take care of themselves, prevention with positives, meaning when they are HIV positive, now they will not infect others." -014
Concerns and Values about Disclosure	
Negatively Impacts the Child	"They feel that the child is going to perform poorly in school or they will not to school, they will stop living, you know, they will stop doing things that they were doing" -011
Child is too Young	"It's the age, you feel that even after 15 (years) the child is still young and they don't know anything. And also they want to cushion, they don't want the child to get hurt with the news." -010 "And then the mother feels guilty so much that she infected the child so she doesn't want those questions. Maybe even the mother is still in denial, or there's still blame that the father brought this disease and once she discloses to the child, she will feel that the memories will come, those bad memories, that now am HIV positive and I infected the child, all this is because of the unfaithfulness of the dad, so they fear such questions from the child. " -012
Inability to Answer Questions from the Child	
Failure to Come to Terms with Own Status	"Some of these caregivers are not yet open and they have not accepted their status, they are still struggling with their own issues of health, HIV, they have still not accepted fully that, I am HIV positive and all this. So to talk to their children and about HIV, it's a topic they don't even want to discuss about." - 009
Disclosure to Child Involves Telling Others	"Even at home we have found that some, maybe the mother of the child, knows her status and the status of the baby but the husband is in the dark, doesn't know that these people are on ARVs, they are HIV positive, and now to bring the issue of disclosure in such a family, it will not happen." -009
Alter Family Relationships and Knowledge	"It means that if you are telling me to tell my child that they are infected, you are telling them that I am also infected. But these are now the biological parents, usually that is their fear." - 015
Blame and Guilt Regarding Transmission	"They don't want to look bad in front of their kids, they don't want their kids to see, I was sexually not upright, I did this and this, I am the cause of the problem." - 006
Stigma/Discrimination Towards the Child and Family	"They say that the community will look at their children differently....because children don't know so many things, they are just comfortable sharing it out with anybody so they feel that the children will share it out to the community and people will start talking about it.....so they feel like the children will be discriminated on." -007
Lack of Knowledge/Information	"When you see maybe it's a very old lady who has brought this child. And maybe they are not even understanding what we are talking about." – 010

Table 2.3: Provider-Identified Triggers Indicating Pathway-Specific Entry Into and Movement through the Disclosure Process

Triggers Used in the Assessment and Decision-Making Process	
Age	
Below 7	"If they are not yet 8, maybe they are 6, 5 (years), we postpone disclosure to a later date but we alert the mother or the caregiver that we might start the disclosure process at the age of 8 or even 7, so as you continue coming to the clinic, please think of how you will be able to start the process." - 011
Age 7-8	"The proper age to start, according to me, is when at least when the child can differentiate good and bad and that is from around 7, 7 years" - 002
Above 8	"It's good for these children to know their status when they are at least 12 (years)". That way you can handle and talk to them but above that, you might not be able to talk to them. They will be out in school, maybe in boarding schools and they will be nowhere to listen to you." - 010
Understanding and Awareness	
Level of Understanding	"Age, we have seen that it cannot help us to know. But understanding, IQ for each and every individual is different. You can have a child who is 10 years and she still doesn't even want to take care of herself, so it is the caregiver or the mother to tell us the level of understanding of the child. About the age, it doesn't matter, it doesn't matter. There are some who even at 6 years, they can ask questions, so that is the correct child to tell, yeah." - 014
Asking Questions	"The first and foremost is when the child starts asking, "why do I take these drugs and am not sick." That also tells you that the child wants to understand what is happening." - 013
Able to Read	"And then the children get to be sent to the laboratory and they read, you know they know how to read, they read the lab request form, they know CD 4, they read about HIV on the form, and they get to understand." - 001
Putting 2 and 2 together	"A child and the caregiver will come in and they'll say, "My child saw this advert on TV and looked at me and asked, "those drugs are taken by people who are HIV and I take the same drugs." Those are the kinds of triggers that we see and we know that it has to be done immediately." - 010
Personality	
Outgoing	"And you know those who come and they are very active, even they are very talkative, and they express themselves so much. For us we know that the child is going to learn about their status quite early" - 011
Curious	"Then when they come here, I will see the mood of the child, how alert she is when talking to the parent about the drugs, do you take the drugs at the right time, I'll say let me examine the child, then I will see the facial expression as a clinician. I can be able to know, this child is alert, she is curious, she wants to know what am I discussing with the parent concerning her health, then now that's the time I will separate and ask and emphasize on disclosure. Then I take the mother for preparation." - 012
Behavior Change	"We ask them (the mother/caregiver), does the kid have mood swings or any behavioral (issues), you know. If you start seeing such then we tell them that it is time. Because some of the kids may never ask by the way, what disturbs them, so you observe, you should be able to observe and know if your child is in their right senses, in their right mood. If you start seeing anything different, then you should start to seek....ask, what is the problem?" - 006

School Transitions	"For example tomorrow they might go into a boarding school or they are in high school and as such, they need to take care of themselves because at this time, there will be no caretaker. So at this time of the hour, they need to continue with their treatment even here as before when the guardian was there." – 013
Social Situations	"He was an orphan, he is an orphan actually. And the aunties didn't actually want to disclose. So when I talked to the aunty and I told her you need to disclose to the child, the aunty said no, no, no, no, no. I cannot disclose because even the time the mother died, the boy was not told that his mother died, so up to date the boy does not know that his mother died, he only knows that his mother travelled somewhere far, yeah. So the aunty actually did not want at all, she said it will traumatize the boy, we cannot disclose." - 012

Table 2.4: Informing the Development of Disclosure Best Practices: Logistical challenges, identified needs and techniques facilitating success

Techniques Facilitating Success	
Involvement of the entire family in the process	"They come with the father plus the kids, the whole family comes, as in they...we even see them together.....apart from when you want to ask about the sexual activities for the kids, you know. And it's so smooth." - 006
Have support and follow-up post-disclosure for children	"I remember there was at time a child told the mother, 'if you know you were HIV positive, why did you have to give birth to me?' Unfortunately the child was mad with the parents, she refused drugs, but she died. So it is that extent, so that is why we decided that these children have to be seen more often, every time they come to the clinic we have to assess them, it doesn't matter whether the adherence is good, we have to assess them, because every time things change." - 014
Include access to peer and mentor support for children	"There is a day we had some doctor mentors who came in and they took three children to mentor, just walk with them career-wise, encourage them. If they have a little pocket money, they give these kids or take them out for picnics once in a while. They came and introduced, now we communicated to the hospital, so we are waiting for them to give us a feedback so that those doctors can come in and introduce, because the kids were so excited about that...that you know, I have a friend who is a doctorIt's something good for them because you know most of them come from very poor families." - 015
Have separate clinic days for adolescents	"It's a new support group because of the challenges that we have with the youth, with the teenagers not taking their medicine well and all these challenges of school, taking their medicine in school in front of their peers and all that, so we have provided that day for them." - 010
Include access to peer support for caregivers	"So all this will bring them together and now, when we bring them together, they share their views. So those who are successful can encourage those who have not succeeded and tell them the benefits, because when it comes from them, it has the impact as opposed to when it come from me, because now this is someone who is on the ground, "I have a child who is HIV positive, I did this and this is what is taking place." - 014
Have caregivers sympathize with the child during disclosure	"I also learned to tell these mothers later on that you should move with the child because.....let the child see that you are also maybe learning this for the first time because if you look strong and he is crying, he is emotionally crying, he feels that you are not with him on that. Feel with him, just be there, let him learn from your facial expression that you are also shocked or you are learning that for the first time, though you know it." - 013
Give providers access to support	"Even as a supervisor, when I am weighed down, my colleagues have to support me ... We meet and we share the client work, the challenges we faced.. we still can have one on one supervision with a peer mentor and we can just sit and just discuss, offload, and then they now give you support and then you get better, so we don't carry this home, by the time we are getting out of this, we have forgotten." – 014
Work as a team to encourage disclosure	"We involve the counselor; the counselor will talk about it and even we can make a home visit, that is the social worker now, and also they should tell us why they don't want to disclose. Depending on the reason they give us, we have team work, we can involve somebody else who can do it best." - 012
Identified Gaps and Needs	

Training to improve comfort and confidence for providers	"So even after training on disclosure, it is still a challenge but because of the skills that I have, we know how to go about it." - 014
Training and materials to support alternative forms of communication	"Maybe I have some good colors, or maybe I have some dolls or maybe I have some pens or paper or something that they can respond through that, because not all children love to talk, sometimes they want to write down or sometimes they want to put something in action. So at that point what happens is that when they come to your room and you have enough tools, now they will communicate to you through the tools that they have." - 014
Guidance on how to best support caregivers	"How we take care of the caregivers. The challenges they have, I think they need to be addressed because they are true, they are right. We keep saying awareness is out for HIV but no, when you talk to some people, the awareness is not yet out properly." - 015
Continued medical education training to keep current with disclosure	"It's always important to keep up to date information so it's always important to have continuous on job training on disclosure issues. Also having time to analyze cases where caregivers come together and give several cases where they went through disclosure successfully or they didn't do it successfully, in such case scenarios now people can be able to learn more." - 016
Development of standardized disclosure guidelines or SOPs	"I think sessions are better off if there is a structured way, like you see, I am just saying it from the way I have been handling the kids. I have experience in counseling, I have gone to some training in counseling which involved a small bit on child counseling, so not like really I'd say on a professional basis I would do this and this but on an experience basis I'll say that I would do this and this, you know." - 004
Standardized National Approach	"We are all scattered, small pockets here and there, but if it was all consolidated into one, so that we have the same information, it would be easier." - 015
National networking for the children	"We want the kids that are also there to come out so that we can come together, share the experiences together, what these other people can feel, so the way they are networked, they are networked and maybe you have a calendar day for the HIV/AIDS day, they come together and share the information and fill their part and now not seeing the HIV as a big challenge, the way it used to be before, but now seeing that now we can still continue being HIV positive, it does not mark the end of life, my kids always tell me that it does not mark the end of life, they usually continue working together as if they were normal" - 005
Logistical Challenges and Barriers	
Rotating or non-consistent counselors	"Remember we are different counselors, we are about five and I can start a process and I am not in, but now my colleague is in and the parent feels that disclosure should be done, the child is ready for disclosure. So what happens, this person has to create a rapport with this child again before the disclosure is done." - 014

Having to watch what is said to the child when disclosure hasn't happened	"I would feel like, when I have seen one person, I would feel like my head would spin because there are so many things, there is so much involvement during that session. Because you are really trying to look for the best words to use, now that I am in both adult clinic and children clinic, so I don't mention HIV, I don't mention AIDS, because if I do and the child don't know their status, it may be a challenge. So you have to structure, you know, use words that are appropriate, especially disclosure having not taken place. So initially it was very involving, very...it was quite a lot of involvement, physically, energy and all that." - 011
Child knows, provider knows child knows, caregiver refuses to disclose	"They know it maybe from books, from magazines, from TV, from internet and you will find that they will keep quiet. They will not even talk to the parent about it, yes. But when they come to the session, it's when they will tell us, "yes I saw my medications, I googled somewhere and saw..." And so the parent is seated there and they are like, my child does not know, but they know." - 010
Inability to reach certain caregivers	"Those who don't come, consistently they are absent, so you are not able to reach to them and have them receive these benefits of hearing other caregivers who have disclosed, sharing how they did it, the outcome of the disclosure, how their children have changed since they disclosed, they don't have now that benefit." - 009
Father as head of household blocking disclosure	"As long as this man is not coming to the clinic and he is the one who makes decisions in that family, disclosure will not happen, so in such families you find that the disclosure process takes longer for the children to be disclosed to." - 009
Inability to disclose without caregiver's consent	"They just tell you, I don't want you to tell the child this and this and that is the end of the story. And since you have empowered them, you don't know where to hit after that. You just live with it, you don't disclose." - 006
Decreased patient volume	"And then, the work load should be less so that we have time with our patients, one by one, yeah. Because at times, workload cannot give me more time, to take time, more time with this caregiver or the mother to tell her about disclosure, so we need more time." – 012

Conclusion.

Although HIV acquisition rates have declined 43% since 2003 [1], the pediatric HIV epidemic is not over. Increasing HIV prevalence among women in Africa and incomplete uptake of prevention of mother-to-child transmission (PMTCT) services have hindered the ability to completely prevent the transmission of HIV. In addition, current research has been unable to fully account for the individual variability underlying HIV acquisition and progression. Research identifying the mechanisms underlying these differences can inform the development of novel therapies and vaccines to prevent infection.

While prevention of infection is the ultimate goal, there is an urgent need to develop effective practices to care for already infected children. With increasing access to antiretroviral treatments, the numbers of children experiencing early HIV-related mortality have significantly declined. As children are surviving longer, we need to learn more about how to most appropriately care for these children.

Genetic variations in immune response proteins that alter protein function or expression level may affect HIV susceptibility and pathogenesis. We found that genetic variations in *TLRs* influence pediatric HIV acquisition and progression; specifically a variant in *TLR9* was associated with increased risk of HIV acquisition and variants in *TLRs 7, 8 and 9* were associated with altered peak HIV RNA levels in infected infants. Several findings from my study were consistent with previous studies in cohorts of Caucasian ancestry [39] [43]. Our finding that the *TLR9* 1635G/A was associated with higher risk of HIV-1 acquisition supported a previously observed trend in an HIV MTCT study in a population of Caucasian ancestry [43]. In contrast, a study evaluating this variant in African heterosexual serodiscordant couples found this variant to be associated with decreased risk of HIV acquisition [55]. Thus, mode of transmission, rather than genetic ancestry and patterns of linkage disequilibrium, may be a more important indicator of the role of specific *TLRs* on HIV acquisition. Intriguingly, my study also found that *TLR9* 1635G (rs352140) was associated with decreased acquisition of HIV but higher peak viral load. This is consistent with a model in which factors that increase immune recognition could decrease acquisition by recruiting effective immune responses and increase viral load by increasing immune activation. Future studies should try to replicate

these findings and further refine the role of the *TLR9* 1635G/A variant on HIV acquisition and progression.

My study demonstrated that SNPs in TLR genes may account for some of the observed variation in HIV acquisition and disease progression [36-41, 93]. However, the functional consequences of these genetic variations remain largely unknown. Future studies should seek to determine the functional consequences of genetic variations in TLRs.

We evaluated genetic variations in TLRs. However, recent studies have shown that TLRs are only one family member of a larger family of pattern recognition receptors (PRRs). Specifically, membrane-free cytosolic PRR families have recently been identified including nucleotide-binding oligomerization domain (NOD)-1 like receptors (NLRs), which recognize different components of bacteria and viruses, retinoic acid-inducible gene-1 (RIG-1)-like receptors that recognize viral RNA, and additional cytosolic PRRs able to detect dsDNA such as the AIM2 inflammasome and DAI receptor [31, 94]. Genetic variations in these PRRs may influence HIV acquisition and progression. However, studies have yet to evaluate the role of these PRRs in HIV specifically.

Understanding the role of the infant innate immune response in elimination and control of HIV may help inform the development of specific treatments and vaccines. Infant HIV infection is associated with faster disease progression than adults [60, 61] yet the reasons for more rapid infant HIV progression are poorly defined. One mechanism underlying increased progression could be their relatively weaker adaptive immune responses [61]. Inducing an effective immune response in infants may require a vaccine approach that exploits cross-talk between adaptive and innate immune responses, eliciting a more rapid and robust immune response to HIV without relying on B and T cell memory [20, 29, 61]. Because TLRs generate immediate non-specific responses to pathogens and may induce the development of effective adaptive immunity, TLR agonists could overcome some challenges of developing effective vaccines for HIV exposed and infected children [20, 61]. Vaccine studies in mice using TLR agonists have shown successful release of TLR-specific pro-inflammatory cytokines and chemokines, increased activation and maturation of DCs, macrophages, NK and NKT cells, and correlated responses with increased *gag*-

specific CTL activity [95-97]. Because of the importance of the innate response in infants, developing a vaccine targeted to interact with the innate immune response could be especially important for preventing acquisition. Future research could evaluate the use of vaccines that harness TLR, and other PRR, immune response pathways.

In addition, better understanding the role of TLRs and other PRRs in pediatric HIV could help develop novel therapies for treating pediatric HIV. Activation of PRR-mediated pathways could be either beneficial or harmful in the setting of HIV. TLR-mediated responses should support cell-mediated immune responses. However, in the case of HIV-1 infection, immune activation and inflammation are associated with viral pathogenesis and disease progression [98] and TLR-initiated inflammatory responses could thus lead to higher HIV viral loads and faster disease progression [21, 28]. In this way activation of PRR pathways could paradoxically help prevent HIV acquisition via innate immune responses, but could also be associated with more rapid disease progression in infants who become infected, as we observed with our *TLR9* variant. Understanding the role of PRRs in HIV pathogenesis could point the way toward novel therapies; for example, treatments that inhibit TLR recognition of HIV-associated PAMPs may slow HIV progression in already infected children.

In addition to understanding more about the biological mechanisms underlying pediatric HIV, there is a need to learn more about how to most appropriately care for HIV-infected children. An emerging issue of importance in pediatric HIV is deciding when and how to disclose the child's HIV status to the child. The majority of research on pediatric HIV disclosure has focused on evaluating rates and correlates of disclosure and assessing psychological or adherence outcomes associated with disclosure. While these studies have provided valuable insight into the low prevalence of disclosure in many resource-limited countries and factors that may be influencing these low rates, they do not provide any practical guidance for providers currently doing disclosure in practice. There are limited studies evaluating the processes being used for disclosure and no studies outlining the challenges and successes that providers who use these processes are experiencing. In addition, there are limited disclosure guidelines. To improve knowledge of disclosure processes being employed in practice, my study evaluated the personal

disclosure experiences of health providers involved in caring for HIV-infected children in Kenya. Information from these interviews was used to characterize the rationale providers are using to make decisions to disclose and the processes currently being used in Kenya. We used this information to describe what an optimal disclosure process might encompass.

To expand upon the information gathered in provider interviews, we recently received a pilot grant to explore children, adolescent and primary caregiver experiences with disclosure in Kenya. To-date, we have conducted in-depth one-on-one interviews with 19 children and adolescents and 20 primary caregivers. We asked children and adolescents to share their personal experiences with disclosure and their feelings and coping since learning their diagnosis. We also asked them to identify whether they found the current disclosure process beneficial and how they might change the process to be more effective and appropriate for children experiencing disclosure in the future. We interviewed primary caregivers who have and have not disclosed the child's status to the child. We asked caregivers who disclosed about the challenges and successes they experienced as part of disclosing to the child and to describe overall experiences with process. We also asked them about their anxiety before and after disclosure, tools or support systems used during the process, and the impact of disclosure on their relationship with the child. For primary caregivers who had not disclosed, we asked them about the barriers preventing disclosure, general views on disclosure, and additional tools or support systems that would be beneficial as they think about when, how and who will disclose to the child. We hope to analyze this information to identify themes related to disclosure from both child and caregiver perspectives. We plan to incorporate this information with provider experiences to arrive at an overall disclosure process that balances the needs of the child, caregiver and provider.

We hope to use this information to develop standardized materials and processes that can be used to effectively and sensitively disclose HIV diagnoses to HIV-infected children in Kenya and other international settings. Disclosing HIV status to HIV positive children is of emerging importance because of the increasing numbers of HIV infected adolescents, the importance of preventing the spread of HIV within the young adult population, and the lack of motivation to take many of the current preventive

measures without knowledge of one's HIV status. In addition, given the widespread nature of this problem in other Southern and Central African countries, it will be important to collaborate and share the results of our study with a larger audience.

Findings of our studies can also be used immediately to generate a practical guide for caregivers and providers involved in the pediatric HIV disclosure process within the clinics we interviewed. The information generated from this project will also serve as preliminary data for future inquiry into HIV prevention methods for HIV-infected adolescents and effective communication of complex and potentially stigmatizing health conditions to children in international settings. This is a unique opportunity to explore the real time experiences of children, caregivers and providers before a larger cohort of children reach this age and to use the existing cohort of HIV positive young adults to optimize disclosure for the future generation.

Stakeholders from almost 200 countries have joined together to strategically target the elimination of new pediatric HIV infections by 2015 [1]. While there have been significant reductions in the occurrence of new HIV infections in children, there is still have a long way to go to reach this goal. Even with the elimination of new infections comes the challenge of caring for a large aging population of individuals who were infected with HIV as children. Therefore, the need for research into this area persists. Research into the biological mechanisms underlying infection and how to appropriately care for infected children will remain important to address the global pediatric HIV epidemic.

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