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Patterns of HIV Resistance in Children Attending an Antiretroviral Clinic in Soweto, South Africa: A Case-Control Study

By

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Declaration

I, Zinhle Vilakazi, declare that this Research Report is my own work. It is being submitted for the Degree of Masters of Medicine in Paediatrics at the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at any other University.

(Signature of the candidate)

29 day of November 2023 in Johannesburg

Dedication

I dedicate this work to my husband and daughter who have supported and journeyed with me through this degree.

Presentations and Publications

The resistance profile findings of this study were presented at the University of the Witwatersrand Paediatric Research Day, 2 December 2021. Title of presentation: "Patterns of HIV resistance in children attending an antiretroviral clinic in Soweto".

The case-control aspect of the study findings were presented at the University of the Witwatersrand Paediatric Research Day, 8 December 2022. Title of presentation: "Factors associated with HIV resistance in children attending an antiretroviral clinic in Soweto, South Africa: a case-control study". This presentation was awarded best Registrar Research Project prize.

Abstract

Background

Exposure to suboptimal serum levels of antiretrovirals (ARVs) places resistance pressure on circulating human immunodeficiency virus (HIV), with consequent emergence of resistance. HIV resistance leads to treatment failure and adverse outcomes. We explored factors associated with the emergence of ARV resistance in children living with HIV (CLWH) attending a treatment clinic in Soweto.

Methods

We reviewed the clinical and laboratory characteristics, and factors associated with ARV resistance in children aged 0 to 15 years of age that were treated at the clinic from 01 January 2011 through 31 December 2020. The Stanford HIV drug resistance database was used to identify HIV drug resistance mutations and generate resistance profiles. Characteristics of children that underwent drug resistance testing (DRT) were compared to those of children who remained virologically suppressed on fist-line ARVs.

Results

During the study period, 7,029 children attended the clinic of which 425 (6.0%) underwent DRT (cases) and 953 (13.6%) remained suppressed on first-line ARVs (controls).

The resistance dataset included 431 resistance tests that were done in 425 children and adolescents that were eligible for the study. Non-nucleoside reverse transcriptase inhibitor (NNRTI) mutations accounted for 50.8% of all mutations, followed by nucleoside reverse transcriptase inhibitor (NRTI) mutations (44.5%) and protease inhibitor (PI) mutations (4.6%).

Cases were significantly older at ARV initiation (81.6 vs 45.2 months), had a higher prevalence of ever being diagnosed with tuberculosis (33.2% vs 27.4%), ever being orphaned (57.6% vs 50.6%) and ever experiencing severe acute malnutrition (SAM) (19.8% vs 11.7%). In all

modelling approaches, SAM was consistently associated with ARV resistance (adjusted odds ratios (aOR) ranging from 3.548 (95% confidence interval (CI) 1.979-6.359) to 6.383 (95% CI, 3.811-10.690)). Increasing baseline CD4 percentage was associated with significantly lower adjusted odds of case-status (aOR ranging from 0.971 (95% CI, 0.953-0.989) to 0.951 (95% CI, 0.931-0.972)). Seventeen (5.6%) cases died, compared to two (0.3%) controls; P<0.001.

Conclusions

Tenuous nutritional status was consistently and significantly associated with the requirement for DRT in this cohort of children and adolescents. Conversely, higher baseline CD4 percentage was associated with control status. Early ARV initiation, to preserve immunological status, and nutritional support throughout the course of clinic attendance may limit the emergence of drug resistance in CLWH.

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Nomenclature

3TC	lamivudine
ADR	acquired drug resistance
ART	antiretroviral therapy
CHBAH	Chris Hani Baragwanath Academic Hospital
CLWH	children living with HIV
DRMs	drug resistance mutations
EFV	efavirenz
FTC	emtricitabine
HIV	human immunodeficiency virus type-1
HSCC	Harriet Shezi Childrens Clinic
IQR	interquartile range
LMIC	low-middle income country
NHLS	National Health Laboratory Service
NNRTI	non-nucleoside reverse transcriptase inhibitors
NRTI	nucleoside reverse transcriptase inhibitors
NVP	nevirapine
PDR	pre-treatment drug resistance
PI	protease inhibitor
PLWH	person living with HIV
PMTCT	prevention of mother-to-child transmission
SD	standard deviation
TDR	transmitted drug resistance
VL	viral load
WHO	World Health Organization

1 LITERATURE REVIEW

Human immunodeficiency virus (HIV) infection remains a major global epidemic. UNICEF estimates that of the 39.0 million people living with HIV (PLWH) worldwide at the end of 2022, 1.5 million of them children between the ages of 0-14[1,2]. In South Africa it is estimated that 7.7 million people were living with HIV in 2021[1], with almost half a million of them being children between the ages of 0-14 years[3]. South Africa currently has the largest antiretroviral therapy (ART) treatment programme in the world since its introduction in 2004, with an estimated 5.7 million people receiving ART[1]. The discovery and roll out of ART has improved the quality of life and life expectancy of PLWH substantially[4].

As with any other antimicrobials, ART used in unfavourable conditions can result in the development of resistance[5]. Surveys conducted by the World Health Organization (WHO) in 10 countries in sub-Saharan Africa between 2012 to 2020, found that up to 10% of adults starting ART have drug resistance mutations (DRMs) to the non-nucleoside reverse transcriptase inhibitor (NNRTI) drug class, and nearly half of infants newly diagnosed with HIV have NNRTI resistant virus before initiating treatment[2]. The prevalence of drug-resistant HIV is high in children under 18 months of age and newly diagnosed with HIV[2].

The WHO defines antiretroviral drug resistance as mutations in the genetic structure of HIV that affects the ability of a specific drug or combination of drugs to block replication of HIV[6]. The goals of ART are to inhibit viral replication, halt the progression of disease to acquired immunodeficiency syndrome (AIDS), and to allow for partial restoration of the immune system[7]. When HIV replicates in the presence of ART, genomic mutations occur which may alter the viral proteins that are targeted by antiretroviral drugs, which leads to different types of mutations, some of which may lead to treatment failure[7]. HIV is particularly prone to developing resistance for a number of reasons, including high levels of circulating virus and rapid and error prone reverse transcription of the genome. The process of reverse transcription is inaccurate, due to the absence of any enzymatic proofreading activity[7]. This leads to each genome having at least one mutation which results in diverse HIV populations, some

of which have a survival advantage especially when conferring resistance to antiretrovirals[7]. All current antiretrovirals used in the treatment of HIV, including newer classes, are at risk of becoming partly or fully inactive because of the emergence of drug-resistant strains[6].

1.1 The evolution of ART and PMTCT regimens in South Africa

The development of antiretroviral treatment began in the 1980s, after the initial isolation of HIV, with nucleoside reverse transcription inhibitors (NRTIs) being the first class of antiretrovirals used in the treatment of HIV/AIDS[8]. NRTIs are not without side effects, and have limited durability. Therefore, other classes of antiretroviral drugs such as NNRTIs and proteases inhibitors (PIs), which had different mechanisms of action against HIV, were developed and used in combination therapy in the 1990s for more effective treatment[8]. The combination of different drug classes proved more effective at suppressing viral replication, and was recommended by the WHO in both adults and children as the pandemic of HIV continued[9]. A key strategy that was identified in controlling the paediatric HIV pandemic was the prevention of mother to child transmission (PMTCT) through the use of ART[9].

In South Africa, there was no ART or prophylaxis available in the public health sector prior to 2002[10]. With the introduction of ART in South Africa, the PMTCT programme was introduced. The PMTCT programme aimed to reduce the transmission of HIV from mothers to their infants and young children, either in utero, through the process of delivery of the newborn, or through breastfeeding. Different antiretroviral prophylactic regimens have been implemented and modified over the years depending on the maternal ART status and the exposure risk to the infant[11–13].

The PMTCT programme has evolved in South Africa from its introduction. In 2002, PMTCT consisted of a single-dose of nevirapine (NVP) administered intrapartum to the mother, and postpartum for the infant. The introduction of Option A, in 2008, saw the addition of zidovudine (AZT) monotherapy to pregnant women from 28 weeks and AZT for the infant, for either 7 or 28 days, depending on the duration of AZT in pregnancy of their mother[10,12]. In

the revision of Option A in 2010, the maternal PMTCT regimen consisted of AZT from 14 weeks' gestation, intrapartum single-dose NVP, 3 hourly AZT during labour, and a postpartum single dose of tenofovir (TDF) and emtracitabine (FTC)[12]. The infant PMTCT regimen was changed to NVP for 6 weeks or longer for infants that were breastfeeding whose mothers were not on lifelong ART[10,12].

In 2013, Option B was introduced into the PMTCT programme. In option B, all pregnant women with HIV were started on triple therapy ART for the duration of pregnancy, with ART stopped postpartum for those who did not meet the life-long ART eligibility criteria[14]. Eligibility criteria for lifelong ART were amended to a CD4 count of <350 cells/ţL or WHO clinical stage 3 or 4 disease for pregnant women[10,14]. Option B was modified in 2015 to option B+, which makes life-long ART accessible to all pregnant women living with HIV, regardless of clinical or immunological staging[13,14]. Option B+ also saw a change to the infant prophylactic regimen, with NVP being given for 6 weeks or extended to 12 weeks if there was high risk maternal HIV exposure[10,14]. In 2019, AZT and NVP were given for 6 weeks for all infants that were considered high risk, with NVP extended for a further 6 weeks for breastfed infants[15].

ART in paediatrics has evolved from NRTI mono-therapy to dual therapy and finally the triple therapy[4,16]. In the early era of ART, an NNRTI backbone regimen was the recommended ART regimen for children living with HIV (CLWH)[4]. However, NNRTIs have fallen out of favour due to high levels of resistance to NNRTIs secondary to their use in PMTCT and their low genetic barrier to developing resistance mutations[17–19]. A meta-analysis found that pre-treatment drug resistance (PDR) prevalence in children in sub-Saharan Africa is very high in PMTCT-exposed and PMTCT-unexposed children, with the prevalence of PDR increasing markedly over time[19]. The pooled PDR prevalence in African children is 4–15 times higher than that reported in adults[2,19].

PI-based ART has remained an important component of management of CLWH in the developing world. From 2010, the WHO recommend paediatric first-line ART regimens that

include a boosted PI, such as Lopinavir/ritonavir (LPV/r), combined with two NRTIs for children under 3 years of age[2]. The CHER trial demonstrated that early treatment of infants with boosted PI-based regimens reduces early infant mortality by 76% and HIV progression by 75%[20]. As of 2019, the paediatric first-line ART regimen has undergone change with the inclusion of integrase inhibitors such as dolutegravir (DTG)[4].

The South African first line ART regimen now recommends AZT, 3TC and NVP for neonates that are less than 4 weeks and under 3kgs. The infant can then be switched to ABC + 3TC and DTG at 4 weeks and if their weight is above 3kgs. The first line treatment for children that are more than 30 kgs and are above the age 10 years is TDF +3TC +DTG (TLD)[21]. The PTMCT regimen that an infant receives is dependent upon the maternal viral load which classifies the infant as either high-risk or low-risk. Infants with low-risk exposure receive NVP for 6 weeks. Those with high-risk exposure receive AZT for 6 weeks and NVP for a minimum of 12 weeks[21].

1.2 Classification of drug resistance

HIV drug resistance is classified into three categories by the WHO: 1) acquired HIV drug resistance (ADR); 2) transmitted HIV drug resistance (TDR); and 3) PDR (pre-treatment HIV drug resistance). ADR (acquired resistance) is the most common type of drug resistance and occurs when HIV replicates in the presence of ART, when drug levels are too low to block replication but high enough to exert a positive selection pressure on the virus[7,22]. TDR (transmitted resistance) is detected among ART naïve persons with no history of antiretroviral drug exposure, and occurs through infection with HIV that has drug resistance mutations (DRMs)[23]. PDR (pre-treatment resistance) refers to resistance that is detected among ART naïve persons at the time of starting ART, or in persons with previous antiretroviral drug exposure initiating or re-initiating first-line ART[23]. PDR is clinically important as it is associated with a poor response to first-line therapy and further accumulation of drug resistance mutations[19].

1.3 ART class mutations

A multi-country analysis from sub-Saharan African found that 785 (54%) of 1450 CLWH (median age 18 months) had PDR to 1 or more antiretroviral agents prior to treatment initiation[18]. The rate at which drug resistance occurs across the different classes of ART is dependent upon the drugs' barriers to resistance. First generation NNRTIs have a low barrier to resistance and the accumulation of resistance-associated mutations is rapid, often occurring within 3 months of virological failure[24]. Second-generation NNRTIs, such as etravirine and rilpivirine, have a higher barrier to resistance and confer only partial cross-resistance to nevirapine and efavirenz[24,25]. PIs have much a higher barrier to resistance and are more robust.

A study which investigated the accumulation of HIV-1 drug resistance after continued virological failure on first line ART in adults and children in sub-Saharan Africa, found that at first virological failure, DRMs were detected in 87% of participants, new DRMs accumulated with an average rate of 1.45 (standard deviation (SD) 2.07) DRM per year, with 0.62 (SD 1.11) NNRTI DRMs and 0.84 (SD 1.38) NRTI DRMs per year, respectively[26]. Predicted antiretroviral susceptibility declined significantly after continued virological failure for all reverse transcriptase inhibitors ($P<0.001$ in all analyses)[26]. Acquired drug resistance patterns were similar in adults and children[26].

DRMs were observed in 48% (205/430) Nigerian CLWH under 18 months of age, with resistance to NNRTI highest (45%), followed by resistance to NRTIs (22%) and PIs (2%)[27].

The prevalence of mutations in different regions of the globe is dependent upon the exposure of patients to different ART classes[28]. A systematic review of 30 studies from LMICs in sub-Saharan African, Asia and South America, found that drug-class specific resistance rates were 88% for NNRTIs, 80% for NRTIs, and 54% for PIs, with these resistance rates similar to those reported for European children[29].

The K103N and M184V mutations, which confer high-level resistance to NVP and EFV and lamivudine (3TC) and emtricitabine (FTC), respectively, are more frequent in South Africa

than in other countries. This is likely because of earlier and/or programmatic use of EFV and 3TC or FTC, and possibly by the earlier introduction of Option B+[14,30].

The prevalent subtype of HIV-1 has a contributory factor to the type of mutations that are seen in different regions. The NNRTI mutation V106M is found most frequently in settings in which HIV-1 subtype C predominates, whereas V106A is more frequently seen in regions with subtype B predominance[28]. V106A in subtype B is mainly associated with resistance to NVP but not EFV, unlike V106M which confers broad NNRTI class cross-resistance[28]. The prevalence of the L65A mutation, which also confers resistance to 3TC, is similar regardless of HIV-1 subtype, although this mutation develops more rapidly in populations infected with subtype C[28].

1.3.1 NNRTI mutations

Due to their low barrier to resistance, NNRTI DRMs frequently occur first in the face of virological failure, as demonstrated in a study of accumulation of DRMs in sub-Saharan adults and children on first-line ART[30]. In one study, more than one DRM was detected in 87.4% of ART-experienced adult and paediatric patients in sub-Saharan Africa, and among these, 83.2% harboured NNRTI resistance mutations[24]. In South Africa, the most frequently detected NNRTI mutations include K103N (38.7%), G190A (21.8%), Y181C (20.2%), V106M (8.4%), K101E (8.4%), E138 (7.6%) and V108I (7.6%). The K103N and G190A mutations are linked with NVP exposure and EFV resistance, while Y181C is associated with NNRTI class resistance[24].

Second generation NNRTIs, such as etravirine and rilpivirine, are also compromised in the presence of NNRTI resistance due to high (20-60%) cross resistance rates[5,24,31]. These high rates of cross resistance limit the use of second generation NNRTIs in patients failing first-line ART regimes that contained an NNRTI[5,24,31].

1.3.2 NRTI mutations

A survey of five African countries (South Africa, Zimbabwe, Uganda, Swaziland and Mozambique) found that NRTI resistance prevalence is considerably lower than NNRTI resistance (8.9% compared to 53.0%)[24]. NRTI resistance mutations were mostly determined by the stavudine (D4T) and 3TC NRTI backbone which was commonly used at the time[24,32]. The most frequent NRTI mutations detected were M184V (69.7%), any thymidine analogue mutation (9.2%), K65R (5.9%) and K70R (5.0%)[24]. The prevalence of K65R mutation, associated with resistance to tenofovir (TDF), was low in Swaziland, Uganda and Mozambique while in South Africa and Zimbabwe, position 65 mutations were more common reflecting the greater use of TDF in maternal regimens in those two countries[24].

M184V mutation is the most frequently encountered mutation to the NRTI class, and compromises the susceptibility of FTC and 3TC, however continued treatment with these NRTIs is not contraindicated as M184V impairs viral replication fitness and increases TDF activity[24,25].

Thymidine analogue mutations (TAMs), which reduce NRTI susceptibility by facilitating primer unblocking, are more prevalent in second-line ART failure[25,31]. This is likely due the longer duration of time on any failing therapy, whether it be first-line or second-line. Of note is that the Q151M pan-nucleoside mutation is infrequently detected in paediatric patients[24].

1.3.3 Protease inhibitor mutations

The prevalence of resistance to PIs in infants and young children newly diagnosed with HIV in sub-Saharan countries is low (<3%), probably due to low rates of maternal PI use[5]. A large, historical cohort of children in Soweto conducted over 10 years, investigating virologic failure on first-line LPV/r-based therapy, reported that only 8/75 (10.7%) children failing LPV/r-based first-line ART had significant PI DRMs, including two with intermediate resistance to darunavir (DRV)[33]. Among 63/75 (84%) children remaining on LPV/r-based therapy, 32/63 (51%) achieved virologic suppression, despite two of these children having significant LPV mutations[33]. The findings of that study attested to the robustness of PIs, even in the face of

prolonged virological failure.

A 2014 study conducted in South Africa of a cohort of 16 children (median age 5 years) who were failing an LPV/r regimen, found a major PI mutation (V82A) in only one child, with the most common pattern for children failing LPV/r being an isolated M184V (i.e. NRTI) mutation[34].

In Southern Africa, LPV/r and full-dose ritonavir are widely used in paediatric regimens. The most frequently reported mutations from this region were V82A, I54V, and M46I, which are selected by LPV/r[28].

In Cape Town, the PI mutations M46I, I54L, L/V82A/F and L90M were observed in a group of children failing full-dose first-line ritonavir-based ART[24].

1.3.4 Integrase strand transfer inhibitor resistance

Since 2018, dolutegravir (DTG) has been recommended by the WHO to be used as first line treatment, with close monitoring for emergence of integrase inhibitor (INSTI) resistance in CLWH failing DTG-based regimens[22]. South Africa introduced DTG with 2 NNRTIs as the first line regimen for CLWH in 2019[21]. Although INSTI resistance has been rare there have now been multiple case reports of INSTI-associated resistance[35]. The most frequently detected INSTI resistance mutations included T66A/I/K, E92G/Q, G118R, F121Y, E138A/K/T, G140A/C/S, Y143C/H/R/S, S147G, Q148H/R/K, N155H, S230R and R263K[35].

1.4 Prevalence of and risk factors for ART drug resistance

The WHO reports that in several low-middle income countries (LMICs), more than 1 in 10 adults starting ART are infected with HIV that is already resistant to efavirenz (EFV) or nevirapine (NVP). In these groups, women were twice as likely as men to carry PDR strains of HIV. This challenges the elimination of mother-to-child transmission of HIV, and impacts on maternal and child health outcomes[6]. Furthermore, 25% persons initiating first-line ART

may have previous exposure to antiretrovirals, either in the form of PMTCT or treatment interruption, and are three times more likely to have PDR to EFV or NVP compared to those with no prior antiretroviral exposure[22].

In surveys conducted in nine sub-Saharan African countries by the WHO in 2012 through 2018, the prevalence of PDR among children under 18 months with newly diagnosed HIV was high: over half of the infants carried strains of HIV that were resistant to EFV and/or NVP[23]. Furthermore, a multi-country analysis reported one in two children newly diagnosed with HIV under the age of 18 months as being infected with an HIV strain resistant to NNRTIs, and therefore at high risk for suboptimal virological response to treatment if initiated on an NNRTI-based regimen[18]. The prevalence of PDR to NRTIs may also exceed 10% in newly diagnosed infants in some countries[7].

Data suggests that PDR is not the only problem affecting children accessing ART. Viral suppression is often not achieved as a consequence of poor adherence to therapy, which increases the risk of accumulating resistance. A systematic review of resistance data in children from LMICs found that 90% of those failing first-line regimens had at least one detectable resistance mutation[29]. Children present with more HIV drug resistance than adults, and have a 2-fold higher risk of virological failure (VF) compared to adults after 5 years on ART[28]. A Meta-analysis of 72 studies that reported on 51,347 CLWH in LMICs, revealed only 73% attained a VL <1000 copies/mL within 12 months of ART initiation[26].

The probability of virological suppression and long-term treatment success is lower in children than in adults. Younger children typically have poorer virological responses than do older children, which is associated with high viral loads before treatment and the risk of sub-therapeutic drug concentrations due to limited paediatric formulations, variable pharmacokinetics, and changes in body weight because of growth[2,29]. An important determinant of poor viral suppression is the continued use of suboptimal ART regimens in children[36]. Other contributing factors for suboptimal ART use in children include unpalatable formulations, high cost of PIs and the scarcity of paediatric-trained clinicians which contributes

to the continued, prolonged use of unfavourable regimes[36]. Established risk factors for virological failure in children include male gender, age, concurrent tuberculosis (TB) treatment, and maternal orphanhood[28]. Multi-class antiretroviral resistance has also been associated with PMTCT exposure, sub-optimal mono- and dual-therapy ART regimens, lack of child-friendly formulations, defaulting ART and limited availability of approved antiretrovirals for children[19].

PMTCT exposure is considered a major risk factor for resistance as it has resulted in an increase in HIV drug resistance among infants and children who acquire HIV infection[18]. A multi-country analysis of five sub-Saharan countries identified that children who had received neonatal prophylaxis independent of maternal prophylaxis were nearly twice as likely to have drug-resistant HIV compared to children who did not receive neonatal prophylaxis, and prevalence of resistance to NNRTIs decreased with increasing age (61.9% in children aged <3 months, but 25.4% in children aged 12–18 months)[18]. Furthermore, a study performed in Uganda found that HIV drug resistance occurred in 35.7% of children with a history of PMTCT exposure, compared to 15.6% in children with unknown PMTCT history and 7.7% among children who were ART naïve[37]. Another Ugandan study Of 701 children found that 34% of the patients failed first line ART, with median time to first line ART failure of 26.4 months (interquartile range (IQR), 18.9 – 39.1 months). Factors associated with treatment failure were poor adherence (odds ratio (OR) 10.0, 95% confidence interval (CI): 6.4–16.7), exposure to single dose NVP as part of PMTCT (OR 4.2, 95% CI: 1.8-9.4) and receipt of a NVP-containing regimen (OR 2.2, 95% CI: 1.4-3.6)[38].

A South African study analysed factors associated with development of antiretroviral resistance mutations in 65 young children (median age 16.8 months) failing PI-based ART, and found major PI mutations in 49% of children. Risk factors associated with these mutations were low weight-for-age and height-for-age, longer duration of PI-containing regimens, virological failure, and unsuppressed HIV viral load at 12 months of ART[39]. Anti-tuberculosis (TB) treatment at the time of ART initiation and use of ritonavir as single PI were also significantly associated with the emergence of PI-resistance[39]. On multivariate analysis, cu-

mulative months on PI-based ART and use of ritonavir as single PI were independently associated with the emergence of PI-resistance[39].

1.4.1 Anti-tuberculosis therapy as a risk factor for PI resistance

Tuberculosis co-infection is a major risk factor for PI DRMs as a result of the drug-drug interaction between LPV/r and rifampicin which is known to reduce plasma concentrations of LPV/r which may lead to virological failure and development of PI DRMs[4].

1.4.2 Malnutrition as a risk factor for ART resistance

Malnutrition has been shown to contribute to the development of resistance. In a study from Mozambique, reduced peak weight-for-age was significantly associated with ART resistance[40]. In the same study, immunosuppression of any form and length of time on ART were associated with the development of antiretroviral resistance[40].

1.5 Future of ART in children - the threat of resistance

Based on the high prevalence of PDR in children[18,19], the WHO currently recommends that all CLWH should have a genotyping resistance test prior to initiation of ART, and if requiring a change to second-line ART to tailor the individual ART regimes[22]. Genotyping is frequently done in high-income countries but in LMICs such as South Africa, routine resistance testing is rarely performed due to the cost and complexities of resistance genotyping, which limits testing to children that are failing second-line regimens[23]. Resource limited regions, where the burden of HIV is most prevalent, rely on large scale treatment programmes with standardised empiric ART regimes[5]; however, surveillance of drug resistance becomes important in resource limited settings to ensure that empiric regimes are appropriate.

In South Africa, the current National Department of Health guidelines recommend resistance testing in children when there is virological failure after more than 2 years of being on a PI- or an integrase inhibitor based regimen[21].

HIV Drug resistance poses a major public health challenge and impacts individual patient

care as it reduces the number of available and effective treatment options and threatens the success of both prevention and treatment programs such as the UNAIDS 95-95-95 global treatment targets[1,41]. Understanding HIV drug resistance in the paediatric population is particularly important because children have higher viral load set points, with more rapid disease progression compared to adults[7].

1.6 Justification for this study

Understanding HIV resistance mutations in a population impacts on decisions around the choice of ART regimens and ensures sustainability and durability of these regimens. Despite South Africa having the world's largest ART programme[17], there is a paucity of data on the prevalence and types of resistance mutations in children, and a need for more studies in this area. The aim of this study is to describe the resistance mutations of children attending a paediatric HIV clinic in a tertiary hospital. Furthermore, this study aims to determine the risk factors associated with certain mutations and the clinical outcomes of those with mutations compared to those without mutations.

2 MANUSCRIPT IN SUBMISSIBLE FORMAT

Abstract

Background

Exposure to suboptimal serum levels of antiretrovirals (ARVs) places resistance pressure on circulating human immunodeficiency virus (HIV), with consequent emergence of resistance. HIV resistance leads to treatment failure and adverse outcomes. We explored factors associated with the emergence of ARV resistance in children living with HIV (CLWH) attending a treatment clinic in Soweto, Johannesburg, South Africa.

Methods

We reviewed the clinical and laboratory characteristics, and factors associated with ARV resistance in children aged 0 to 15 years of age that were treated at the clinic from 01 January 2011 through 31 December 2020. The Stanford HIV drug resistance database was used to identify HIV drug resistance mutations and generate resistance profiles. Characteristics of children that underwent drug resistance testing (DRT) were compared to those of children who remained virologically suppressed on fist-line ARVs.

Results

During the study period, 7,029 children attended the clinic of whom 425 (6.0%) underwent DRT (cases) and 953 (13.6%) remained suppressed on first-line ARVs (controls).

The resistance dataset included 431 resistance tests that were done in 425 children and adolescents that were eligible for the study. Non-nucleoside reverse transcriptase inhibitor (NNRTI) mutations accounted for 50.8% of all mutations, followed by nucleoside reverse transcriptase inhibitor (NRTI) mutations (44.5%) and protease inhibitor (PI) mutations (4.6%).

Cases were significantly older at ARV initiation (81.6 vs 45.2 months), had a higher prevalence

of ever being diagnosed with tuberculosis (33.2% vs 27.4%), ever being orphaned (57.6% vs 50.6%) and ever experiencing severe acute malnutrition (SAM) (19.8% vs 11.7%). In all modelling approaches, SAM was consistently associated with ARV resistance (adjusted odds ratios (aOR) ranging from 3.548 (95% confidence interval (CI) 1.979-6.359) to 6.383 (95% CI, 3.811-10.690)). Increasing baseline CD4 percentage was associated with significantly lower adjusted odds of case-status (aOR ranging from 0.971 (95% CI, 0.953-0.989) to 0.951 (95% CI, 0.931-0.972)). Seventeen (5.6%) cases died, compared to two (0.3%) controls; P<0.001.

Conclusions

Tenuous nutritional status was consistently and significantly associated with the requirement for DRT in this cohort of children and adolescents. Conversely, higher baseline CD4 percentage was associated with control status. Early ARV initiation, to preserve immunological status, and nutritional support throughout the course of clinic attendance may limit the emergence of drug resistance in CLWH.

Introduction

Human immunodeficiency virus (HIV) infection remains a major global epidemic. South Africa currently has the largest antiretroviral therapy (ART) programme, with 7.7 million people living with HIV, and half a million children living with HIV (CLWH)[6,42]. ART used in unfavourable conditions can result in the development of drug resistant mutations (DRMs)[6]. All current antiretroviral drugs, including newer classes, are at risk of becoming partly or fully inactive because of the emergence of drug-resistant strains[6].

Surveys conducted by the World Health Organization (WHO) found up to 10% of adults starting HIV treatment may have drug resistance to the non-nucleoside reverse transcriptase inhibitor (NNRTI) drug class[2]. Of even greater concern is that one in two children newly diagnosed with HIV infection under the age of 18 months are infected with a strain that is resistant to NNRTIs, and are therefore at high risk for suboptimal virological response to treatment if initiated on an NNRTI-based regimen[18]. Children present with more HIV drug resistance than adults, and have a 2-fold higher risk of virological failure (VF) compared to adults after 5 years on ART[31]. The probability of virological suppression and long-term treatment success is lower in children than in adults[31].

A critical determinant of poor viral suppression in children is the continued use of suboptimal ART regimens[27]. Factors contributing to suboptimal ART use in children include unpalatable formulations, high cost of protease inhibitors (PIs) and the scarcity of paediatric-trained clinicians which contributes to the continued, prolonged use of unfavourable regimens[27]. A study from Pretoria, South Africa, reported male gender, age, concurrent tuberculosis (TB) treatment, and maternal orphanhood as being independently associated with virological failure[28]. Furthermore, immunosuppression of any form and longer duration of ART are significantly associated with the development of drug resistance[19].

Multi-class antiretroviral resistance in the paediatric population has also been associated with prevention of mother-to-child transmission (PMTCT) exposure, suboptimal mono- and dual-therapy ART regimens, default off ART, and limited availability of approved antiretrovirals

for children[5]. Furthermore, malnutrition is a risk factor for the development of resistance[39].

In this study, we aimed to explore the prevalence of antiretroviral drug resistance, patterns of resistance, and risk factors for resistance in a cohort of children and adolescents attending a large public sector ART clinic in Johannesburg, South Africa.

Methods

We present a retrospective case-control study of paediatric patients under the age of 15 years who were tested for HIV resistance from 01 January 2011 to 31 December 2020.

Inclusion criteria

Inclusion criteria for cases (children who were tested for HIV resistance) were age <15 years, with any history of ART exposure prior to submission of the resistance test sample.

For analysis of potential risk factors associated with ART class mutations among children who underwent HIV resistance testing, children and adolescents that had resistance testing but in whom the ART class mutations were not detected, were assigned to the control group. For analysis of potential risk factors associated with virologic resistance, children and adolescents that were virologically suppressed on first-line ART and therefore never underwent resistance testing were assigned as being the control group. Children and adolescents failing third line ART, and those without HIV resistance testing although they were virologically unsuppressed, were excluded from the analysis.

Data sources

The Harriet Shezi Children's Clinic is located at Chris Hani Baragwanath Academic Hospital (CHBAH), a public sector academic hospital in Soweto, South Africa. The clinic was established in 2000 and has served over 9,000 CLWH (personal communication, Dr Sipambo). From 2013 onwards, the clinic has focused on the outpatient management of complicated CLWH, serving as a referral site for surrounding clinics and hospitals. Prior to 2018, HIV drug resistance testing was performed for all children failing first line (NNRTI- or PI-based),

second line, or third line ART regimens. From September 2018 onwards, drug resistance testing was no longer performed on those failing first line NNRTI-based regimens.

These changes allowed the clinic to align its HIV resistance testing algorithm with National Department of Health recommendations. Demographic, clinical and laboratory data were extracted from the clinic's REDCap database[43,44]. HIV drug resistance test results were obtained, with permission, from the National Health Laboratory Service (NHLS).

Definitions

Virologic failure was defined as a plasma HIV viral load (VL) >1000 RNA copies/mL based on two consecutive viral load measurements 3 to 6 months apart, with the first VL at least 6 months after ART initiation. Timing of ART initiation was summarised as an ordinal categorical variable, with "early", "early middle", "late middle" and "late" periods defined as ART initiation prior to 2004, from 2004 through end-2009, from 2010 through end-2014 and from 2015 through end-2020, respectively.

Analysis

Exploratory data analysis was conducted to evaluate the clinical and laboratory characteristics of cases and controls during the study time period. Continuous variables were evaluated for normality of distribution, and described as mean and standard deviation (SD) for normally distributed variables and median and interquartile range (IQR) for skewed variables. Categorical variables were summarised as proportions. The Student t test was used to compare means between groups, and the Wilcoxon sign rank test was used to compare medians.

HIV drug resistance mutations were evaluated using the Stanford University on-line drug resistance database for all sequences submitted to the NHLS during the study time period[31,45].

Five strategies were used for the case-control analysis, including four approaches to case-control matching using the MatchIt package in R[46]. The unmatched analysis compared the characteristics of cases and controls using logistic regression, adjusting for age at ART

initiation and era in which ART was initiated. In matched analyses, coarsened exact matching with and without replacement within strata, nearest neighbour matching, and full matching were employed, adjusting for age at ART initiation and era in which ART was initiated in all analyses. Once cases were matched to controls, the characteristics of cases and controls in the weighted strata were compared using logistic regression.

All logistic regression was conducted as univariate models initially, and variables that were significantly associated with case-status in univariate analyses were combined in the multivariable model for each case-control analysis. Potential risk factors that were considered in the case-control analyses included sex, age at HIV diagnosis, age at ART initiation, first line ART regimen used (NNRTI- or PI-containing), exposure to PMTCT, baseline CD4 count, baseline HIVVL, previous or current anti-tuberculosis treatment, malnutrition, and orphan status.

All analyses were done using R statistical software, version 4.3.1[47].

Sample Size

A sample size of at least 360 cases (children with HIV drug resistance testing performed) and 360 controls (children suppressed on first-line ART) was required to detect an odds ratio for risk factors of at least 2.0, assuming high correlation between case and control parameters in univariate analyses ($= 0.60$), with 80% power at the 5% significance level.

Ethics

The study was approved by the Human Research Ethics Committee of the University of the Witwatersrand (clearance number: M210526) and the Ethics Committee of the NHLS (clearance number: PR2119005).

Results

From 01 January 2011 to 31 December 2020, there were 638 resistance tests conducted on 624 children and adolescents living with HIV that attended Harriet Shezi Children's Clinic. Twenty-five children and adolescents were excluded from the analysis as age at ART initiation (n=21) and duration of ART therapy at time of the resistance test (n=4) could not be determined. A further 182 children were excluded, as they were over 15 years of age at the time of the resistance test. The resistance dataset included 431 resistance tests that were done in 425 children and adolescents, and analysis included only the first resistance test from each patient (n=425).

Resistance Analysis

Characteristics of children that underwent HIV resistance testing

The median age at ART initiation in children that underwent ART resistance testing was 82.5 months (IQR, 27.7 to 112.8 months; Table 2.1). Half (52.2%) of the children that underwent resistance testing were males. Baseline values for absolute CD_4 count, Viral load were taken at initiation of ART. The median absolute CD4 count at baseline was 350 cells/uL (IQR, 123.3 cells/uL to 728.5 cells/uL), and the median HIVVL at baseline was 4.85 $\log_{10}$ RNA copies/mL (IQR 3.83 to 5.56) (Table 2.1). Although most of the baseline characteristics were similar between groups, those on ART for a shorter duration of time were significantly older than those that were on ART for longer at the time of resistance testing (96.4 vs. 72.6 months; Table 2.1).

Parameters at the time of resistance testing

At the time of resistance testing, the median age was 130.1 months (IQR, 73.8 to 159.5 months). We stratified the children that underwent resistance testing according to the median time on ART prior to a DRT test being performed. The median time on ART prior to DRT was 33.4 months. The children and adolescents that were on a shorter duration of

ART (< 33.4 months) at the time of resistance testing being significantly younger (114.4 vs. 143.6 months; Table 2.1). Children who were in the shorter ART duration group also had significantly lower CD4 percentages (18.0% vs. 21.6%) and higher HIVVL (4.51 vs. 4.11 $\log_{10}$ copies/mL) at the time of resistance testing (Table 2.1). At the time of resistance testing, 36.9% (143/425) of the children were on PI-containing ART regimens, with a smaller proportion of those in the shorter ART duration group being on PI-containing ART at the time of the resistance test (30.2% vs. 43.2%; P=0.010) (Table 2.1).

Table 2.1: Characteristics of children that underwent HIV resistance testing, stratified by duration on ART at the time of resistance testing

	Overall	ART duration <33.4 months	ART duration >=33.4 months	P-value
n	425	212	213	
Baseline Parameters				
Median age [months, IQR] at ART initiation	82.52 [27.65, 112.80]	96.37 [28.36, 130.80]	72.62 [25.74, 100.59]	<0.001
Male (%)	222 (52.2)	111 (52.4)	111 (52.1)	1.000
Median CD4 count [cells/uL, IQR] at baseline	353.50 [123.75, 725.50]	326.00 [115.00, 653.00]	394.00 [159.00, 811.00]	0.099
Median CD4 percentage [%, IQR] at baseline	13.00 [6.80, 20.38]	12.95 [5.84, 20.42]	13.00 [7.04, 20.13]	0.924
Baseline immunologic status (%)				0.335
None	68 (16.2)	28 (13.5)	40 (18.8)	
Mild	45 (10.7)	26 (12.6)	19 (8.9)	
Advanced	35 (8.3)	16 (7.7)	19 (8.9)	
Severe	272 (64.8)	137 (66.2)	135 (63.4)	
Median HIVVL [log10 RNA copies/mL, IQR] at baseline	4.85 [3.84, 5.56]	4.93 [3.77, 5.60]	4.76 [3.86, 5.48]	0.151
Parameters at Resistance Test				
Median age [months, IQR] at resistance test	130.07 [73.80, 159.45]	114.41 [48.44, 144.30]	143.62 [109.40, 164.96]	<0.001
Median ART duration [months, IQR] at resistance test	33.41 [17.60, 58.84]	17.57 [10.89, 25.03]	58.84 [42.81, 79.84]	-
Mean WFA Z-score (SD) at resistance test	-1.05 (1.22)	-1.13 (1.36)	-0.96 (1.02)	0.574
Mean HFA Z-score (SD) at resistance test	-1.92 (1.19)	-1.97 (1.27)	-1.88 (1.14)	0.619
Median WFH Z-score [IQR] at resistance test	0.42 (1.34)	0.50 (1.62)	0.31 (0.78)	0.576
Median BMI Z-score [IQR] at resistance test	-0.20 (1.30)	-0.12 (1.57)	-0.25 (1.07)	0.509
On PI-based regimen at time of resistance test (%)	143 (36.9)	57 (30.2)	86 (43.2)	0.010
Median CD4 count [cells/uL, IQR] at resistance test	559.50 [321.50, 938.50]	514.00 [217.50, 1006.50]	597.00 [380.00, 878.50]	0.379
Median CD4 percentage [%, IQR] at resistance test	19.49 [12.91, 27.78]	17.99 [10.43, 26.57]	21.60 [15.71, 29.39]	0.010
Mean HIVVL (log10 RNA copies/mL, SD) at resistance test	4.32 (0.84)	4.51 (0.84)	4.11 (0.79)	<0.001
Last Follow-up Parameters				
Median age [months, IQR] at last visit	172.86 [132.30, 210.58]	150.31 [94.21, 180.81]	188.13 [156.66, 216.80]	<0.001
Median ART duration [months, IQR] at last visit	86.26 [50.98, 127.27]	45.52 [29.44, 80.05]	109.97 [81.32, 151.72]	<0.001
Median CD4 count [cells/uL, IQR] at last follow-up	621.50 [342.25, 922.75]	696.00 [422.00, 1070.50]	532.00 [299.00, 817.00]	<0.001
Median CD4 percentage [%, IQR] at follow-up	23.38 [15.05, 30.70]	23.62 [15.03, 30.83]	22.55 [15.11, 30.64]	0.619
Median HIVVL [log10 RNA copies/mL, IQR] at follow-up	2.61 [1.69, 4.09]	2.32 [1.69, 3.88]	3.00 [1.69, 4.25]	0.017

Note:
ART = antiretroviral therapy; BMI = body mass index; HFA = height-for-age; HIVVL = HIV viral load in whole blood; IQR = interquartile range; PI = protease inhibitor; SD = standard deviation; WFA = weight-for-age; WFH = weight-for-height.Baseline immunologic status using CDC CD4 percentage immunologic staging. Last follow up paramaters from the last clinic visit of the cohort prior to extraction of the data. P-values are derived from comparison of groups: Chi-square test or Fisher's exact test, as appropriate, for categorical variables; Student t test for comparison of means; Kruskal-Wallis test for comparison of medians; stratification is by whether the patient was on ART for shorter or longer than the median duration of ART (33.4 months) at the time of the resistance test.

Resistance mutations detected

In the 431 resistance tests that were done, non-nucleoside reverse transcriptase inhibitor (NNRTI) mutations accounted for 50.8% of all mutations, followed by nucleoside reverse transcriptase inhibitor (NRTI) mutations (44.5%) and protease inhibitor (PI) mutations (4.6%).

NRTI mutations were detected in 320 (75.3%) of the children who underwent HIV resistance testing. NNRTI mutations and PI mutations were detected in 259 (60.9%) and 19 (4.5%) of the children who underwent HIV resistance testing, respectively.

The top mutations according to ART class are presented in Figure 2.1.

The most frequent NNRTI mutations detected were K103N (n=154, 20.6%), V106M (n=102, 13.6%), G190A (n=43, 5.7%), P225H (n=40, 5.3%) and V179D (n=35, 4.7%). The top five NNRTI mutations accounted for 374 (49.9%) of all 749 NNRTI mutations.

The most frequent NRTI mutations included M184V (n=314, 47.8%), L74V (n=75, 11.4%), Y115F (n=55, 8.4%), K65R (n=22, 3.4%), and any thymidine analogue mutation (n=20, 3%), these five mutations accounted for 486 (74.1%) of all 656 NRTI mutations.

There were few PI mutations (n=68) overall, the top five of which were M46I (n= 17, 25%), V82A (n=14, 20.6%), I54V (n= 12,176%), L76V (n=7, 10.3%) and I47IV (n=4, 5.9%). These five mutations accounted for 76.5% of all PI mutations (n=52/68). The detected major mutations were M46I, V82A and I47I/V. The remainder of the top five PI mutations were minor mutations.

Factors associated with mutations in children and adolescents that underwent HIV resistance testing

Increasing age at ART initiation was significantly associated with NRTI mutations among children and adolescents that underwent HIV resistance testing (adjusted odds ratio (aOR) 1.055; 95% confidence interval (CI), 1.036 to 1.075) in the nearest neighbour matched model which included 105 cases with NRTI mutations and 105 without NRTI mutations (Appendix

Table 2.6). Initiation of ART in the late period was associated with a significantly lower adjusted odds of NRTI mutations compared to initiation in the early period (aOR 0.61; 95% CI, 0.036 to 0.103) using a model which used a full matching approach (320 cases compared to 105 controls) (Appendix Table 2.6). Similarly, the development of NNRTI mutations was significantly associated with increasing age at ART initiation (aOR 1.044; 95% CI, 1.033 to 1.055) whereas ART initiation in the late era was protective (aOR 0.056; 95% CI, 0.032 to 0.99) (Appendix Table 2.8).

Each month increase in age at ART initiation was associated with a 2.5% (95% CI, 1.1 to 4.1%) reduced adjusted odds of PI resistance mutations in the unmatched model, while ART initiation in the late period (aOR 18.936; 95% CI, 8.328 to 43.057) was associated with PI mutations in the fully matched model (Appendix Table 2.10). Factors such as malnutrition, orphan status and prior tuberculosis therapy were not associated with the development of ART class resistance mutations in these analyses.

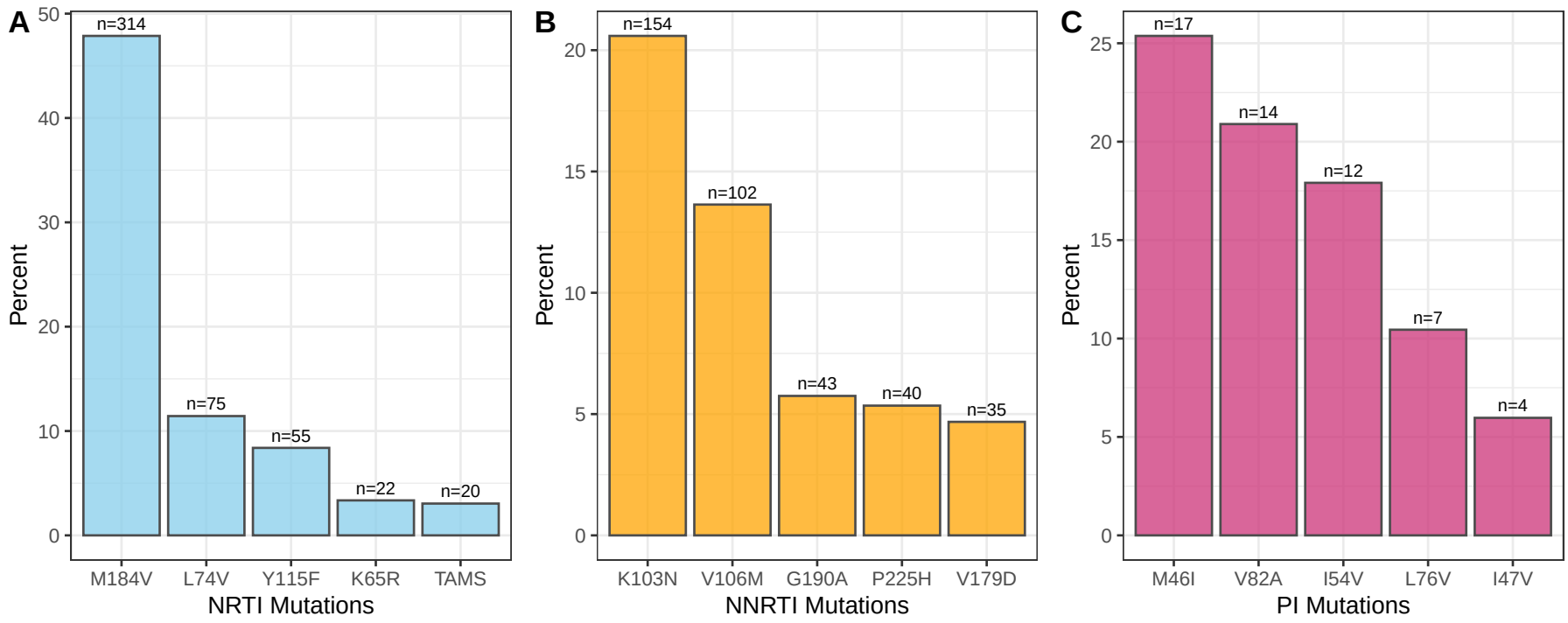

Figure 2.1: The 'top 5' mutations per ART class detected in children and adolescents attending the Harriet Shezi Children's Clinic, 2011 through 2020

NRTI = nucleoside reverse transcriptase inhibitor; NNRTI = non-nucleoside reverse transcriptase inhibitor; PI = protease inhibitor; TAMS = thiamine analogue mutations (M41L, L210W, T215Y (Type 1 TAMS); D67N, K70R, T215F, K219Q/E (Type 2 TAMS)).

Parameters at the time of last follow-up in children and adolescents that underwent HIV resistance testing

At last follow-up clinical assessment , children and adolescents who were on ART for a shorter time period prior to a resistance testing were significantly younger (150.3 vs. 188.1 months), had been on ART for a shorter time period (45.5 vs. 110.0 months), had higher CD4 absolute counts (696 vs. 532 cells/uL) and a better virologic response to change in ART therapy (HIVVL 2.32 vs. 3.00 $\log_{10}$ copies/mL) compared to those that had been on ART for a longer time period at the time of the resistance test (Table 2.1).

Predictors of ART class mutations

Multiple matching strategies were employed in a multivariable logistic regression to identify risk factors for the different ART class mutations amongst the children that had a resistance test (Table 2.7, table 2.9, table 2.11, Supplementary tables).

In the unmatched comparison, initiating ART at an older age (P<0.001) and an unknown PMTCT history (P=0.046) were associated with NNRTI resistance mutations. In the nearest neighbour matching, older ART initiation age was significantly associated with NNRTI and NRTI resistance mutations (P<0.001 for both comparisons). Children that were initiated on ART after 2010 (late middle to late ART era) had increased risk of both NNRTI and NRTI mutations as demonstrated in the full matching comparison.

Risk factors for PI mutations were associated with initiating ART at a younger age in the unmatched comparison (P=0.001) and children that initiated on ART in the early middle era (2004-2010) to the late ART era (2015 onwards).

Case-control analysis

Case-Control Analysis of Factors Associated with HIV Resistance

Table 2.2 summarises the characteristics of the cohort of CLWH that were treated during the study time period (7,029 children and adolescents), as well as those of the cases (n=425),

controls suppressed on first-line ART (n=953), and CLWH that were not included in the main case-control analysis (n=5,651). Controls were significantly younger at ART initiation (45.2 months; IQR, 13.3 to 91.8 months) compared to cases (81.6 months; IQR, 28.4 to 112.8 months); P<0.001 (Table 2.2). Controls had significantly better immunological status at baseline than did cases, but had similar baseline HIVVL results (Table 2.2). Eighty-one percent of the controls were initiated onto ART in the "early middle" period (2004 to end-2009), whereas almost half of the cases (46.9%) were initiated onto ART in that time period. A significantly greater proportion of cases compared to controls were ever diagnosed with tuberculosis (33.2% vs. 27.4%; P=0.034), ever orphaned (57.6% vs. 50.6%; p=0.018), or were ever severely malnourished (19.8% vs. 11.7%; 0.001) (Table 2.2). All cases that experienced severe acute malnutrition were malnourished prior to the resistance test.

Table 2.2: HIV Clinic Database for Cases (children that underwent resistance testing) and Controls (children that did not have resistance testing done)

	Overall	Cases	Controls Suppressed on First-line ART	P-value	Other Clinic Attendees
n	7029	425	953		5651
Male (%)	3540 (50.4)	222 (52.2)	471 (49.4)	0.365	2847 (50.5)
Median age at ART initiation (months) [IQR]	48.82 [13.00, 101.17]	81.61 [28.42, 112.78]	45.18 [13.30, 91.81]	<0.001	46.97 [12.21, 101.20]
Median CD4% at baseline [IQR]	16.38 [9.52, 24.80]	13.77 [7.05, 20.44]	16.63 [10.57, 24.00]	<0.001	16.60 [9.56, 25.20]
Median CD4 count at baseline [IQR]	537.00 [245.00, 1020.00]	381.00 [162.00, 762.75]	566.00 [294.00, 1019.00]	<0.001	544.00 [245.50, 1036.50]
Baseline immunologic status (%)				0.039	
None	1185 (18.2)	75 (17.7)	176 (18.5)		934 (18.2)
Mild	979 (15.0)	43 (10.1)	133 (14.0)		803 (15.6)
Advanced	725 (11.1)	42 (9.9)	122 (12.8)		561 (10.9)
Severe	3620 (55.6)	264 (62.3)	522 (54.8)		2834 (55.2)
Log10 HIVVL at baseline [IQR]	4.92 [3.69, 5.67]	4.89 [3.91, 5.58]	4.93 [3.94, 5.70]	0.523	4.92 [3.58, 5.68]
ART Initiation Era				<0.001	
Early (<2004)	84 (1.2)	2 (0.5)	8 (0.8)		74 (1.3)
Early Middle (2004 to end-2009)	3832 (54.6)	196 (46.9)	772 (81.0)		2864 (50.7)
Late Middle (2010 to end-2014)	1991 (28.4)	167 (40.0)	108 (11.3)		1716 (30.4)
Late (2015 to end-2020)	1115 (15.9)	53 (12.7)	65 (6.8)		997 (17.6)
Ever diagnosed with TB (%)	1751 (24.9)	141 (33.2)	261 (27.4)	0.034	1349 (23.9)
TB diagnosed pre-resistance test (%)	1733 (24.7)	123 (28.9)	261 (27.4)	0.597	1349 (23.9)
Ever orphaned (%)	2695 (38.3)	245 (57.6)	482 (50.6)	0.018	1968 (34.8)
Orphaned pre-resistance test (%)	2673 (38.0)	223 (52.5)	482 (50.6)	0.554	1968 (34.8)
PMTCT (%)				0.061	
Not received	3774 (53.7)	239 (56.2)	562 (59.0)		2973 (52.6)
Received	1241 (17.7)	63 (14.8)	169 (17.7)		1009 (17.9)
Unknown	2014 (28.7)	123 (28.9)	222 (23.3)		1669 (29.5)
Ever SAM (%)	564 (13.4)	82 (19.8)	73 (11.7)	0.001	409 (12.9)
SAM pre-resistance test (%)	564 (13.4)	82 (19.8)	73 (11.7)	0.001	409 (12.9)
Median duration on ART (months) [IQR]	38.10 [19.06, 68.00]	79.81 [44.86, 119.35]	54.67 [42.71, 71.19]	<0.001	31.30 [15.72, 62.40]
Died (%)	108 (2.5)	17 (5.6)	2 (0.3)	<0.001	89 (2.6)

Note:
ART = antiretroviral therapy; HIVVL = HIV viral load in whole blood; IQR = interquartile range; PMTCT = prevention of mother-to-child transmission; SAM = severe acute malnutrition; TB = tuberculosis. P-values are derived from comparison of Cases (children with HIV resistance testing done) and Controls that were suppressed on First-line ART: Chi-square test or Fisher's exact test, as appropriate, for categorical variables; Kruskal-Wallis test for comparison of medians.

Multivariable analysis of factors associated with case-status

Severe acute malnutrition was consistently associated with case-status in each of the multivariate logistic regression analyses, with aORs ranging from 3.548 (95% CI, 1.979 to 6.359) to 6.383 (95% CI, 3.811 to 10.690) (Table 2.3). Increasing age at ART initiation was significantly associated with HIV resistance testing in the unmatched and fully matched models, with each one month increase in age being associated with a 2.7 to 3.9% increased adjusted odds of case-status (Table 2.3).

ART initiation in the late era (2015 onwards) was associated with significantly reduced adjusted odds of case-status in the unmatched and nearest neighbour matched models (Table 2.3). Baseline immunological status discriminated between case and control status in the coarsened exact matched models, nearest neighbour matched model, and fully matched models with increasing CD4 percent or absolute count associated with control-status (Table 2.3).

While all of the models indicated an association between orphan status prior to resistance testing and case-status (aOR ranging from 1.228 to 1.937), this was statistically significant in the nearest neighbour matched model only. Although treatment for tuberculosis prior to resistance testing was associated with case-status in univariate analysis using coarsened exact matching without replacement (OR 1.45; 95% CI, 1.04 to 2.01, Supplementary Table 2.14), it was not associated with case-status in any of the multivariable models (Table 2.3).

Receipt of PMTCT was significantly associated with case-status in the fully matched model (aOR 1.746; 95% CI, 1.003 to 3.042) but safeguarded against cases-status in the coarsened exact matched model (aOR 0.535; 95% CI, 0.334 to 0.857) (Table 2.3).

Table 2.3: Multivariable Logistic Regression Outputs: Unmatched and Matched Case-Control Models

Parameter	Adjusted Odds Ratio	Lower Est	Upper Est	P-value
Unmatched				
Intercept	0.073	0.037	0.137	<0.001
ART start age	1.039	1.033	1.045	<0.001
Early Middle (Yes)	6.093	1.580	27.393	0.01
Late Middle (Yes)	0.522	0.169	1.444	0.221
Late (Yes)	0.479	0.284	0.837	0.007
TB pre-resistance test (Yes)	1.121	0.788	1.591	0.524
Orphaned pre-resistance test (Yes)	1.228	0.855	1.766	0.266
PMTCT (Yes)	1.206	0.759	1.911	0.425
PMTCT (Unknown)	1.040	0.698	1.550	0.847
Ever SAM (Yes)	5.519	3.502	8.752	<0.001
Coarsened Exact Matching without replacement				
Intercept	2.529	1.595	4.009	<0.001
PMTCT (Yes)	0.532	0.332	0.852	0.009
PMTCT (Unknown)	0.661	0.430	1.014	0.058
Baseline CD4%	0.971	0.954	0.989	0.002
TB pre-resistance test (Yes)	0.916	0.618	1.356	0.66
Orphaned pre-resistance test (Yes)	1.547	1.028	2.328	0.037
Ever SAM (Yes)	3.527	1.964	6.335	<0.001
Coarsened Exact Matching with replacement				
Intercept	3.449	1.135	10.481	0.029
PMTCT (Yes)	0.812	0.484	1.361	0.428
PMTCT (Unknown)	0.892	0.555	1.434	0.637
Baseline CD4%	0.978	0.950	1.007	0.131
Baseline CD4 count	0.999	0.999	1.000	0.003
Baseline log10 HIVVL	0.865	0.735	1.017	0.079
TB pre-resistance test (Yes)	0.667	0.416	1.068	0.092
Orphaned pre-resistance test (Yes)	1.387	0.871	2.206	0.168
Ever SAM (Yes)	4.809	2.846	8.126	<0.001

Table 2.3: Multivariable Logistic Regression Outputs: Unmatched and Matched Case-Control Models *(continued)*

Parameter	Adjusted Odds Ratio	Lower Est	Upper Est	P-value
Nearest Neighbour Matching				
Intercept	10.878	3.819	30.986	<0.001
Early Middle (Yes)	6.635	2.200	20.012	0.001
Late Middle (Yes)	0.455	0.199	1.042	0.063
Late (Yes)	0.552	0.340	0.896	0.016
PMTCT (Yes)	0.582	0.320	1.057	0.075
PMTCT (Unknown)	0.683	0.438	1.067	0.094
Baseline CD4%	0.952	0.931	0.972	<0.001
Baseline log10 HIVVL	0.766	0.656	0.894	0.001
TB pre-resistance test (Yes)	0.958	0.629	1.460	0.843
Orphaned pre-resistance test (Yes)	1.881	1.254	2.820	0.002
Ever SAM (Yes)	4.237	2.278	7.882	<0.001
Full Matching				
Intercept	0.212	0.058	0.776	0.019
ART start age	1.030	1.024	1.037	<0.001
Early Middle (Yes)	10.161	2.120	48.711	0.004
Late Middle (Yes)	0.294	0.087	0.996	0.049
Late (Yes)	0.734	0.395	1.365	0.329
PMTCT (Yes)	1.315	0.700	2.473	0.394
PMTCT (Unknown)	1.172	0.741	1.856	0.497
Baseline CD4%	0.969	0.942	0.997	0.033
Baseline CD4 count	1.000	1.000	1.000	0.751
Baseline log10 HIVVL	0.957	0.831	1.101	0.538
TB pre-resistance test (Yes)	1.232	0.836	1.817	0.292
Orphaned pre-resistance test (Yes)	0.952	0.617	1.469	0.825
Ever SAM (Yes)	6.521	3.959	10.741	<0.001

Note:

ART = antiretroviral therapy; HIVVL = HIV viral load in whole blood; PMTCT = prevention of mother-to-child transmission; TB = tuberculosis; SAM = severe acute malnutrition.

Survival analysis

Of 304 cases that underwent resistance testing whose outcome status was known, 17 (5.6%) were known to have died during the study time period. Among controls, 2 (0.3%) of 623 with known outcomes died. Kaplan-Meier estimates of survival indicated significantly better survival outcomes in controls (Figure 2.2).

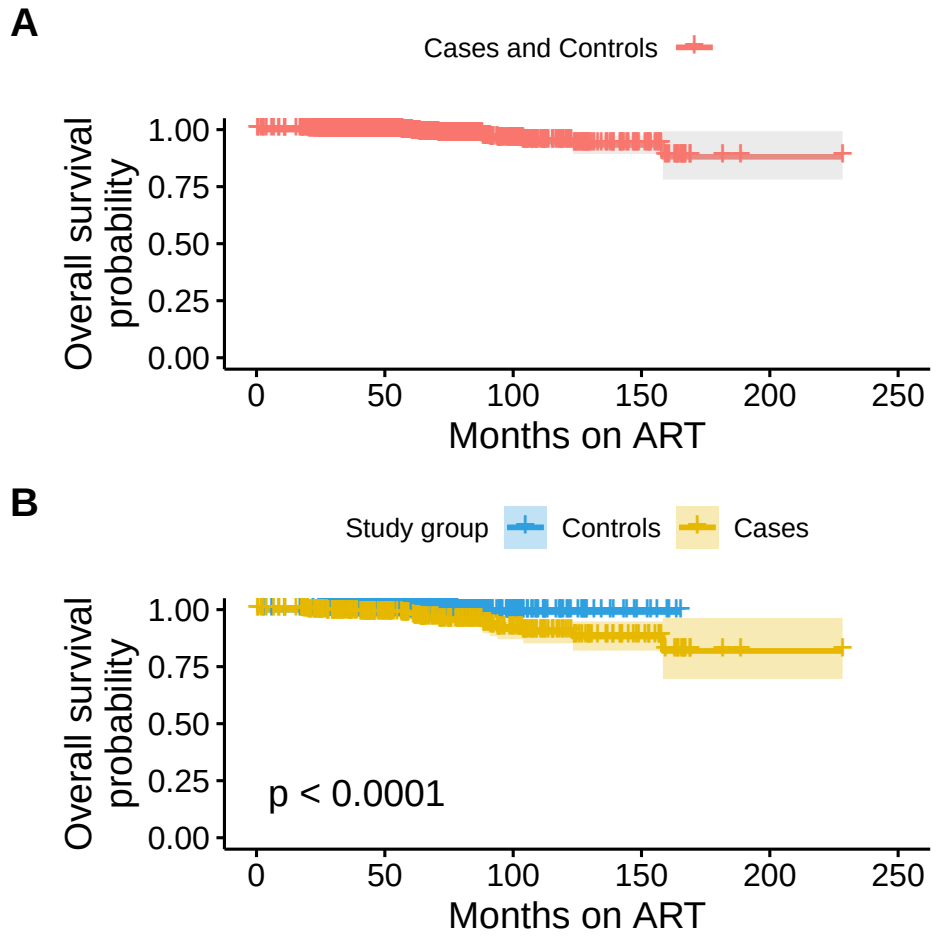

Figure 2.2: Kaplan-Meier survival curves for cases and controls

A = combined survival curve; B = Stratified by study group (cases were children and adolescents that underwent HIV resistance testing, controls were clinic attendees that were virologically suppressed on first line ART).

Discussion

In this study, we reviewed the prevalence of antiretroviral DRMs and factors that possibly predict the development of ART resistance mutations in children and adolescents under the age of 15 years, attending a large public sector ART clinic in Johannesburg, South Africa.

Mutations to NNRTIs accounted for the majority of the mutations, followed by NRTI mutations and lastly PI mutations, similar to other studies in the region[5,24,29]. In low and middle income countries like South Africa, 90% of children failing first line regimens have at least one detectable resistance mutation at time of virological failure[29].

The rate at which these mutations accumulate was not investigated in our cohort however in a study published in 2016 including Sub-Saharan African children and adults, found DRMs accumulated at an average rate of 1.45 DRM per year[24].

The M184V mutation was the most frequent mutation detected in our cohort, followed by K103N mutation. The prevalence of mutations in different settings is dependent upon the exposure of patients to different ART classes[31]. The K103N and M184V mutations are more frequent in South Africa than in other sub-Saharan countries, and likely arise due to earlier and/or programmatic use of efavirenz and lamivudine or emtricitabine, and possibly by the earlier introduction of Option B+ which resulted in life-long ART being accessible to all pregnant women living with HIV, regardless of clinical or immunological staging as a PMTCT strategy[14,30].

Mutations of NNRTIs accounted for the majority of mutations in the cohort. This is expected as the rate at which drug resistance develops across the different classes of ART is dependent upon the drug's genetic barriers to resistance. First generation NNRTIs have a low barrier to resistance and the accumulation of resistance-associated mutations is rapid, often occurring within 3 months of virological failure[24]. The high proportion of children in this study with NNRTI mutations could also represent the pre-treatment drug resistance as a result of ART exposure from PMTCT or the transmission of a resistant virus from the mother[22,24] .

The top five NNRTI mutations K103N, V106M, G109A, P225H and V179D in our study were similar to those found in other South African cohorts[5,24,48].

The K103N mutation is associated with reduced susceptibility to NVP and EFV. V106M, a mutation commonly detected in subtype C viruses, also results in resistance to NVP and EFV. V179D reduces the susceptibility of both first generation NNRTI (NVP, EFV) and to second generation NNRTIs such as etravirine (ETR) and rilpivirine by 2-3 fold[31]. G109A reduces susceptibility to NVP 50-fold, whilst its effect on EFV is less[31]. The P225H mutation in combination with K103N results in a more than 50-fold reduction in susceptibility to NVP and EFV, and a 5- to 10-fold reduction in doravirine (DOR) susceptibility[31]. The mutation Y181C, which is associated with resistance to all NNRTIs, was present in our cohort though not as regularly as in other studies in similar settings[24].

The finding of mutations to second generation NNRTIs in our cohort further supports the current thinking of the limited efficacy of NNRTIs in future regimens. The development and regular use of newer drug classes, such as integrase inhibitors and second generation PIs, is imperative in second and third line regimens in resource limited settings that rely on standardised empiric ART treatment.

The NRTI mutations detected in the cohort were similar to what was found in other sub-Saharan studies[5,24,49]. The M184V mutation, which confers resistance to 3TC and FTC, was the most frequent mutation detected, occurring in 72.9% (341/431) of the DRTs performed. However the presence of M184V is not a contraindication to the use of 3TC/FTC, as the mutation results in impaired viral fitness and increases susceptibility to AZT and TDF[7].

The mutations L74V and Y115F select for resistance to ABC. The combination of L74V and M184V is a common pattern of mutation in patients on an ABC/3TC regimen which was the recommended first line NRTI paediatric combination since the replacement of d4T with ABC due to the unfavourable side effect profile of d4T[5,14].

The K65R mutation, associated with TDF and ABC resistance, was found in only 5.1% (22/431) of the DRTs. The low levels of TDF resistance in this cohort, are likely due to TDF

being prescribed infrequently in children and the use of TDF being endorsed in children in this cohort who are older than 10 years and weigh more than 35 kilograms[15]. This finding supports the use of TDF in future regimens as recommended by the current WHO guidelines[2].

The Type 1 TAMs found in this cohort, individually and in combination, result in high level resistance to AZT and D4T. The Type 2 TAMs, D72N and K70R, also result in high level resistance to mainly AZT and D4T[31].

The Q151M complex, a pan-nucleotide mutation that confers high level resistance, was not present in the cohort. This is similar to what was found in other paediatric cohorts, unlike adult patients were the Q151M mutation is frequently detected[24]. This is likely due to Q151M being selected by TDF, which is not used as frequently in children compared to adults.

The proportion of children that were on a PI regimen that underwent resistance testing in the study was 31% (139/435). The prevalence of resistance mutations to PIs in this cohort was low at 4.6%, which attests to the robustness of this drug class. In Southern Africa, the most frequently reported PI mutations are V82A, I54V, and M46I, which are selected by lopinavir and ritonavir[31] as detected in this study.

Factors associated with resistance to NRTIs and NNRTIs were similar, and included older age at initiation of ART, an unknown PMTCT history and initiating on ART beyond 2010. These are likely due to PMTCT being more available and guideline changes to longer duration on NVP/AZT for the infant and widespread availability of ART to pregnant women. Initiating PIs at a younger age was a risk factor for PI mutations and this may be due to developing metabolic pathways, variability in pharmacokinetics, the unpalatable taste of PI's and issues with the storage of Protease inhibitors[39]. In a sub-analysis of the 26 children and adolescents with any PI mutation(s), half (53.8%), had 3 to 5 PI mutations detected in their DRTs. Clinical characteristics of children with 1 or 2 PI mutations were similar to those with 3 to 5 PI mutations.

The WHO ART guidelines currently recommend dolutegravir (DTG) with 2 NRTIs as first line and as second line in children and adults. DTG in combination with NRTIs is effective,

even in patients with extensive NRTI resistance[50].

There were 953 children that were suppressed on first line that matched to the cases that underwent DRT. CD4 percentage, CD4 count, older age at ART initiation, tuberculosis (TB), orphan status, nutritional status and duration of ART have an impact in the likelihood of having a DRT performed. Children that underwent resistance testing were older at time of initiation of ART compared to the controls. This could be due to older children being initiated on an Efavirenz (NNRTI) regimen, unlike the younger children that would be initiated on the more robust PI based regimen as per guidelines in place at the time. The control cohort had higher CD4 counts and percentages at baseline and this is likely due to the cohort being younger and therefore having higher CD4 absolute counts. The probability of survival diminishes with the longer duration of ART.

A diagnosis of TB increased the likelihood of case-status in our analysis, although not statistically significant in the case-control multivariate outputs. High pill burden impairing adherence and the effects of rifampicin on cytochrome P450 activity, which affected the metabolism of PI's, may be factors associated with the requirement for DRT in children failing ART[7].

Malnutrition was associated with the development of resistance across all models of comparison, this is similar to other studies performed in Southern Africa[39,40]. All of the children with SAM in this cohort had SAM prior to resistance testing. Malnutrition predisposes children to resistance by changing the pharmacokinetics of drugs through altered metabolic function, body composition and intestinal absorption of ART[39,40]. This highlights the importance of the nutritional status of children living with HIV. Ensuring that children are well nourished with food aid programmes, social grants and the involvement of dietitians/nutritionists in the management of children who are on ART should be considered in preventing resistance. There are few studies that interrogate malnutrition as a risk factor for resistance and more studies should be done to investigate the impact of interventions that improve the nutritional status of children on ART and the risk of resistance development.

HIV Drug resistance poses a major public health challenge and impacts individual patient care as it reduces the number of available and effective treatment options and threatens the success of both prevention and treatment programmes such as the UNAIDS 95-95-95 global treatment targets. Routine HIV genotyping is not feasible in resource-limited regions where large scale treatment programmes with standardised empiric ART regimens are used to treat persons living with HIV. Continued surveillance and monitoring of resistance patterns is important to ensure sustainability of ART treatment programmes.

Conclusions

Surveillance of HIV drug resistance and the predictors of resistance development are important in resource-limited settings to ensure that empiric regimes are appropriate and sustainable. The WHO ART guidelines currently recommend dolutegravir (DTG) with 2 NRTIs as first line and as second line in children and adults. DTG in combination with NRTIs is effective, even in patients with extensive NRTI resistance[50].

Substandard nutritional status was consistently and significantly associated with the requirement for DRT in this cohort of children and adolescents. Conversely, higher baseline CD4 percentage was associated with control status. Early initiation of ART to preserve immunological status, and nutritional support throughout the course of ART clinic attendance may limit the emergence of drug resistance in CLWH.

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APPENDIX 1: Supplementary Tables

Table 2.4: Characteristics of children that underwent HIV resistance testing, stratified by age at ART initiation

	Overall	ART start <82.1 months	ART start >=82.1 months	P-value
n	425	212	213	
Baseline Parameters				
Median age [months, IQR] at ART initiation	82.52 [27.65, 112.80]	26.84 [10.35, 57.89]	112.80 [98.68, 132.54]	-
Male (%)	222 (52.2)	112 (52.8)	110 (51.6)	0.882
Median CD4 count [cells/uL, IQR] at baseline	353.50 [123.75, 725.50]	598.00 [271.50, 1036.50]	205.00 [51.00, 451.00]	<0.001
Median CD4 percentage [%, IQR] at baseline	13.00 [6.80, 20.38]	14.55 [8.61, 23.88]	11.00 [3.61, 18.00]	<0.001
Baseline immunologic status (%)				0.045
None	68 (16.2)	28 (13.3)	40 (19.0)	
Mild	45 (10.7)	30 (14.3)	15 (7.1)	
Advanced	35 (8.3)	20 (9.5)	15 (7.1)	
Severe	272 (64.8)	132 (62.9)	140 (66.7)	
Median HIVVL [log10 RNA copies/mL, IQR] at baseline	4.85 [3.84, 5.56]	4.99 [3.90, 5.78]	4.71 [3.76, 5.28]	0.003
Parameters at Resistance Test				
Median age [months, IQR] at resistance test	130.07 [73.80, 159.45]	73.72 [44.65, 123.05]	154.33 [136.42, 168.66]	<0.001
Median ART duration [months, IQR] at resistance test	33.41 [17.60, 58.84]	39.15 [21.35, 73.97]	28.36 [15.97, 48.65]	<0.001
Mean WFA Z-score (SD) at resistance test	-1.05 (1.22)	-1.00 (1.16)	-1.34 (1.54)	0.424
Mean HFA Z-score (SD) at resistance test	-1.92 (1.19)	-1.90 (1.12)	-1.93 (1.27)	0.870
Median WFH Z-score [IQR] at resistance test	0.33 [-0.13, 1.00]	0.32 [-0.12, 0.98]	0.77 [-0.23, 1.16]	0.895
Median BMI Z-score [IQR] at resistance test	-0.10 [-0.96, 0.60]	0.12 [-0.78, 0.82]	-0.38 [-1.21, 0.21]	0.008
On PI-based regimen at time of resistance test (%)	143 (36.9)	110 (56.4)	33 (17.1)	<0.001
Median CD4 count [cells/uL, IQR] at resistance test	559.50 [321.50, 938.50]	925.00 [551.00, 1372.00]	379.00 [159.00, 597.00]	<0.001
Median CD4 percentage [%, IQR] at resistance test	19.49 [12.91, 27.78]	24.94 [17.83, 31.66]	16.22 [9.38, 22.72]	<0.001
Mean HIVVL (log10 RNA copies/mL, SD) at resistance test	4.32 (0.84)	4.28 (0.84)	4.35 (0.85)	0.542
Last Follow-up Parameters				
Median age [months, IQR] at last visit	172.86 [132.30, 210.58]	144.52 [88.84, 185.09]	190.66 [167.82, 223.97]	<0.001
Median ART duration [months, IQR] at last visit	86.26 [50.98, 127.27]	96.97 [61.34, 152.00]	78.57 [44.68, 112.36]	<0.001
Median CD4 count [cells/uL, IQR] at last follow-up	621.50 [342.25, 922.75]	758.50 [516.75, 1174.00]	446.00 [198.50, 708.25]	<0.001
Median CD4 percentage [%, IQR] at follow-up	23.38 [15.05, 30.70]	26.91 [19.05, 33.31]	18.98 [11.01, 26.32]	<0.001
Median HIVVL [log10 RNA copies/mL, IQR] at follow-up	2.61 [1.69, 4.09]	2.42 [1.69, 3.91]	2.80 [1.70, 4.43]	0.054

Note:
ART = antiretroviral therapy; BMI = body mass index; HFA = height-for-age; HIVVL = HIV viral load in whole blood; IQR = interquartile range; NNRTI = non-nucleoside reverse transcriptase inhibitor; PI = protease inhibitor; SD = standard deviation; WFA = weight-for-age; WFH = weight-for-height. P-values are derived from comparison of groups: Chi-square test or Fisher's exact test, as appropriate, for categorical variables; Student t test for comparison of means; Kruskal-Wallis test for comparison of medians; stratification is by whether the patient was older or younger than the median age at initiation of ART (82.1 months).

Table 2.5: Characteristics of children that underwent HIV resistance testing, stratified by regimen

	Overall	Not on NNRTI-containing ART	On NNRTI-containing ART	P-value
n	425	149	239	
Baseline Parameters				
Median age [months, IQR] at ART initiation	82.52 [27.65, 112.80]	22.77 [7.66, 84.06]	97.52 [59.81, 125.65]	<0.001
Male (%)	222 (52.2)	82 (55.0)	122 (51.0)	0.509
Median CD4 count [cells/uL, IQR] at baseline	353.50 [123.75, 725.50]	499.00 [199.00, 1012.00]	298.00 [91.00, 640.50]	<0.001
Median CD4 percentage [%, IQR] at baseline	13.00 [6.80, 20.38]	14.15 [8.32, 22.62]	12.00 [5.49, 19.98]	0.012
Baseline immunologic status (%)				0.015
None	68 (16.2)	13 (8.8)	48 (20.3)	
Mild	45 (10.7)	20 (13.5)	22 (9.3)	
Advanced	35 (8.3)	16 (10.8)	18 (7.6)	
Severe	272 (64.8)	99 (66.9)	148 (62.7)	
Median HIVVL [log10 RNA copies/mL, IQR] at baseline	4.85 [3.84, 5.56]	4.90 [4.05, 5.74]	4.75 [3.67, 5.41]	0.044
Parameters at Resistance Test				
Median age [months, IQR] at resistance test	130.07 [73.80, 159.45]	80.81 [38.80, 154.33]	138.60 [108.81, 162.48]	<0.001
Median ART duration [months, IQR] at resistance test	33.41 [17.60, 58.84]	37.01 [21.62, 67.85]	30.42 [18.39, 53.60]	0.031
Mean WFA Z-score (SD) at resistance test	-1.05 (1.22)	-1.01 (1.41)	-1.12 (1.08)	0.727
Mean HFA Z-score (SD) at resistance test	-1.92 (1.19)	-1.95 (1.22)	-1.91 (1.20)	0.855
Median WFH Z-score [IQR] at resistance test	0.42 (1.34)	0.38 (1.45)	0.48 (1.28)	0.778
Median BMI Z-score [IQR] at resistance test	-0.20 (1.30)	-0.07 (1.42)	-0.26 (1.28)	0.389
On PI-based regimen at time of resistance test (%)	143 (36.9)	140 (94.0)	3 (1.3)	<0.001
Median CD4 count [cells/uL, IQR] at resistance test	559.50 [321.50, 938.50]	734.00 [454.00, 1328.00]	513.00 [260.50, 721.25]	<0.001
Median CD4 percentage [%, IQR] at resistance test	19.49 [12.91, 27.78]	22.50 [14.99, 30.86]	19.21 [12.10, 25.84]	0.013
Mean HIVVL (log10 RNA copies/mL, SD) at resistance test	4.32 (0.84)	4.27 (0.89)	4.33 (0.79)	0.604
Last Follow-up Parameters				
Median age [months, IQR] at last visit	172.86 [132.30, 210.58]	167.46 [91.93, 206.02]	178.75 [144.63, 216.21]	0.004
Median ART duration [months, IQR] at last visit	86.26 [50.98, 127.27]	95.74 [63.77, 149.05]	84.16 [48.31, 121.62]	0.009
Median CD4 count [cells/uL, IQR] at last follow-up	621.50 [342.25, 922.75]	714.50 [358.75, 1183.00]	572.00 [332.00, 830.00]	0.001
Median CD4 percentage [%, IQR] at follow-up	23.38 [15.05, 30.70]	25.15 [15.14, 32.17]	22.12 [15.73, 29.52]	0.129
Median HIVVL [log10 RNA copies/mL, IQR] at follow-up	2.61 [1.69, 4.09]	3.20 [1.81, 4.51]	2.35 [1.69, 3.80]	0.003

Note:
ART = antiretroviral therapy; BMI = body mass index; HFA = height-for-age; HIVVL = HIV viral load in whole blood; IQR = interquartile range; NNRTI = non-nucleoside reverse transcriptase inhibitor; PI = protease inhibitor; SD = standard deviation; WFA = weight-for-age; WFH = weight-for-height. P-values are derived from comparison of groups: Chi-square test or Fisher's exact test, as appropriate, for categorical variables; Student t test for comparison of means; Kruskal-Wallis test for comparison of medians; stratification is by whether the patient was on an NNRTI-containing regime or not at the time of the resistance test.

Table 2.6: Matching for case-control analysis of risk factors associated with NRTI mutations in children and adolescents that underwent ART resistance testing

	Means Cases	Means Controls	Std Mean Diff	Var Ratio	eCDF Mean	eCDF Distance	Pair Dist
Unmatched: 320 Cases, 105 Controls							
Distance	0.8	0.7	0.2	0.9	0.1	0.1	-
ART Year	2010.0	2009.3	0.2	1.2	0.0	0.1	-
ART Age	77.7	67.9	0.2	0.9	0.1	0.1	-
Coarsened Exact Matching without replacement: 102 Cases, 102 Controls							
ART Year	2009.2	2009.2	0.0	1.0	0.0	0.0	0.1
ART Age	69.8	68.0	0.0	1.0	0.0	0.1	0.1
Coarsened Exact Matching with replacement: 261 Cases, 103 Controls							
ART Year	2009.2	2009.1	0.0	1.0	0.0	0.0	0.1
ART Age	76.2	76.3	0.0	1.0	0.0	0.1	0.1
Nearest Neighbour Matching: 105 Cases, 105 Controls							
Distance	0.8	0.7	1.3	0.1	0.4	0.7	1.3
ART Year	2011.2	2009.3	0.5	0.9	0.1	0.2	1
ART Age	130.2	67.9	1.3	0.1	0.4	0.7	1.3
Full Matching: 320 Cases, 105 Controls							
Distance	0.8	0.8	0.0	1.0	0.0	0.0	0
ART Year	2010.0	2009.1	0.2	1.3	0.0	0.2	1
ART Age	77.7	77.6	0.0	1.0	0.0	0.0	0

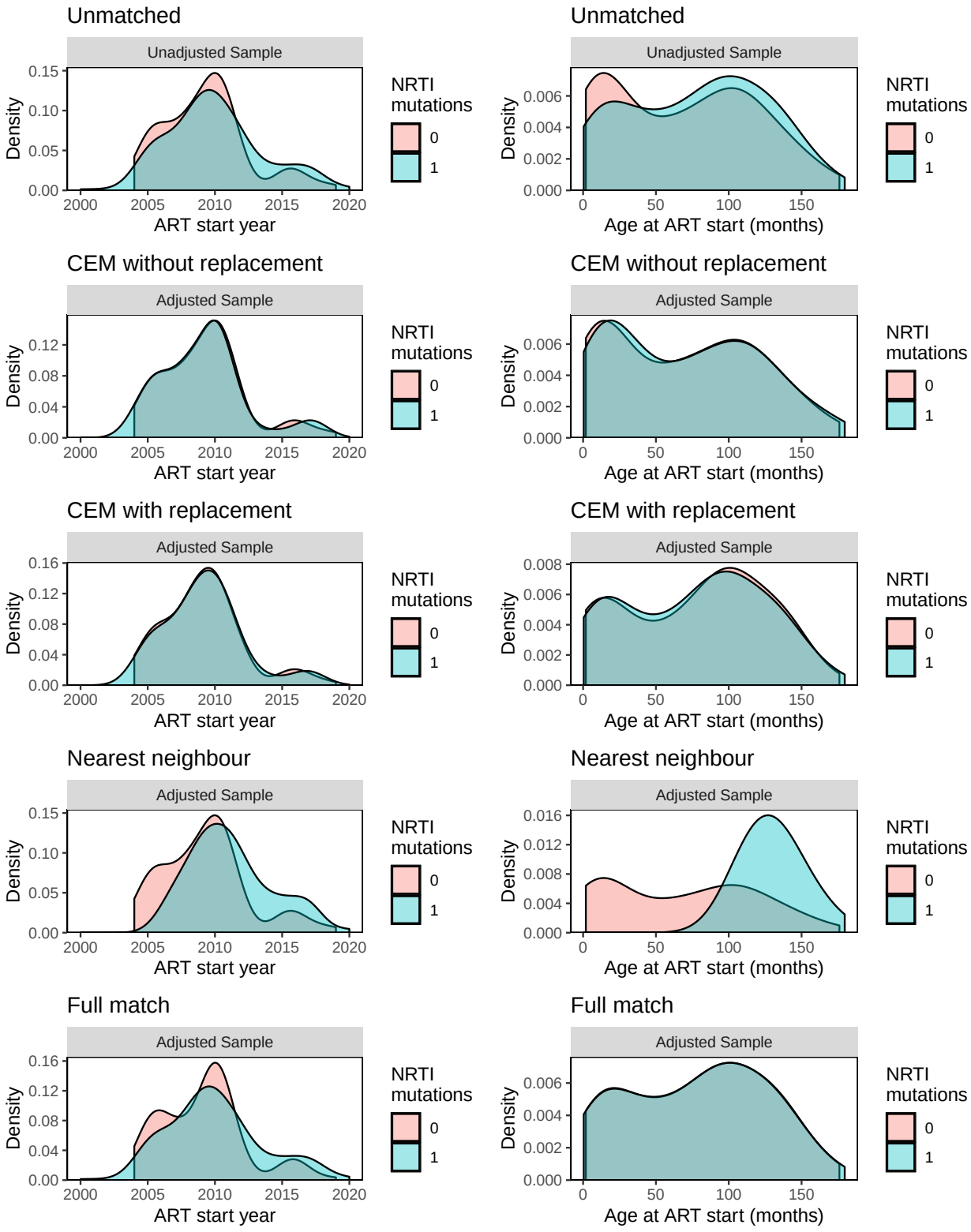

Figure 2.3: Distributional balance for ART start age and ART start age for NRTI resistance mutation analysis

CEM = coarsened exact matching; NRTI = nucleoside reverse transcriptase inhibitor

Table 2.7: Multivariable Logistic Regression Outputs comparing cases with and without NRTI resistance mutations: Unmatched and Matched Case-Control Models

Parameter	Adjusted Odds Ratio	Lower Est	Upper Est	P-value
Unmatched				
Intercept	NE	NE	NE	-
ART start age	1.004	0.998	1.009	0.227
Early Middle (Yes)	NE	NE	NE	-
Late Middle (Yes)	NE	NE	NE	-
Late (Yes)	NE	NE	NE	-
TB pre-resistance test (Yes)	0.842	0.513	1.398	0.5
Orphaned pre-resistance test (Yes)	0.973	0.572	1.643	0.918
Received PMTCT (Yes)	0.979	0.483	2.034	0.953
Received PMTCT (Unknown)	1.117	0.643	1.973	0.697
Ever SAM (Yes)	1.055	0.591	1.937	0.86
Coarsened Exact Matching without replacement				
Intercept	1.238	0.636	2.41	0.529
ART start age	0.999	0.995	1.003	0.731
Early Middle (Yes)	1.122	0.691	1.821	0.641
Late Middle (Yes)	1.065	0.811	1.398	0.652
TB pre-resistance test (Yes)	0.856	0.46	1.595	0.625
Orphaned pre-resistance test (Yes)	1.034	0.548	1.951	0.917
PMTCT (Yes)	0.857	0.358	2.051	0.729
PMTCT (Unknown)	0.691	0.333	1.436	0.322
Ever SAM (Yes)	1.275	0.587	2.766	0.54
Coarsened Exact Matching with replacement				
Intercept	3.004	1.276	7.076	0.012
ART start age	0.999	0.992	1.007	0.88
Early Middle (Yes)	0.88	0.379	2.043	0.765
Late Middle (Yes)	0.996	0.549	1.809	0.99
TB pre-resistance test (Yes)	1.143	0.612	2.134	0.675
Orphaned pre-resistance test (Yes)	0.758	0.4	1.438	0.396
PMTCT (Yes)	0.959	0.419	2.199	0.922
PMTCT (Unknown)	0.929	0.456	1.892	0.839

Table 2.7: Multivariable Logistic Regression Outputs comparing cases with and without NRTI resistance mutations: Unmatched and Matched Case-Control Models *(continued)*

Parameter	Adjusted Odds Ratio	Lower Est	Upper Est	P-value
Ever SAM (Yes)	0.885	0.41	1.908	0.755
Nearest Neighbour Matching				
Intercept	NE	NE	0.022	-
ART start age	1.055	1.036	1.075	<0.001
Early Middle (Yes)	0.765	0.287	2.038	0.592
Late Middle (Yes)	0.974	0.467	2.029	0.943
TB pre-resistance test (Yes)	0.855	0.334	2.186	0.743
Orphaned pre-resistance test (Yes)	1.109	0.465	2.643	0.815
PMTCT (Yes)	2.352	0.672	8.228	0.181
PMTCT (Unknown)	1.224	0.477	3.141	0.674
Ever SAM (Yes)	0.375	0.111	1.267	0.114
Full Matching				
Intercept	112.904	54.75	232.827	<0.001
ART start age	0.999	0.995	1.002	0.515
Early Middle (Yes)	NE	NE	0	-
Late Middle (Yes)	880.415	369.676	2096.785	<0.001
Late (Yes)	0.054	0.033	0.091	<0.001
TB pre-resistance test (Yes)	0.857	0.46	1.597	0.627
Orphaned pre-resistance test (Yes)	0.874	0.451	1.695	0.691
PMTCT (Yes)	0.943	0.432	2.058	0.883
PMTCT (Unknown)	1.234	0.661	2.304	0.509
Ever SAM (Yes)	0.625	0.309	1.267	0.192

Table 2.8: Matching for case-control analysis of risk factors associated with NNRTI mutations in children and adolescents that underwent ART resistance testing

	Means Cases	Means Controls	Std Mean Diff	Var Ratio	eCDF Mean	eCDF Distance	Pair Dist
Unmatched: 260 Cases, 165 Controls							
Distance	0.7	0.5	1.0	0.7	0.2	0.4	-
ART Year	2010.0	2009.5	0.1	1.4	0.0	0.1	-
ART Age	89.7	52.6	0.9	0.7	0.2	0.4	-
Coarsened Exact Matching without replacement: 106 Cases, 106 Controls							
ART Year	2009.2	2009.2	0.0	1.0	0.0	0.0	0.2
ART Age	71.6	71.3	0.0	1.0	0.0	0.1	0.1
Coarsened Exact Matching with replacement: 206 Cases, 106 Controls							
ART Year	2009.3	2009.2	0.0	1.0	0.0	0.1	0.1
ART Age	87.1	87.3	0.0	1.0	0.0	0.1	0.1
Nearest Neighbour Matching: 165 Cases, 165 Controls							
Distance	0.8	0.5	1.6	0.1	0.4	0.7	1.6
ART Year	2010.4	2009.5	0.3	1.3	0.1	0.1	1
ART Age	116.1	52.6	1.5	0.2	0.4	0.7	1.5
Full Matching: 260 Cases, 165 Controls							
Distance	0.7	0.7	0.0	1.0	0.0	0.0	0
ART Year	2010.0	2009.0	0.3	1.5	0.1	0.1	1
ART Age	89.7	89.6	0.0	1.0	0.0	0.0	0

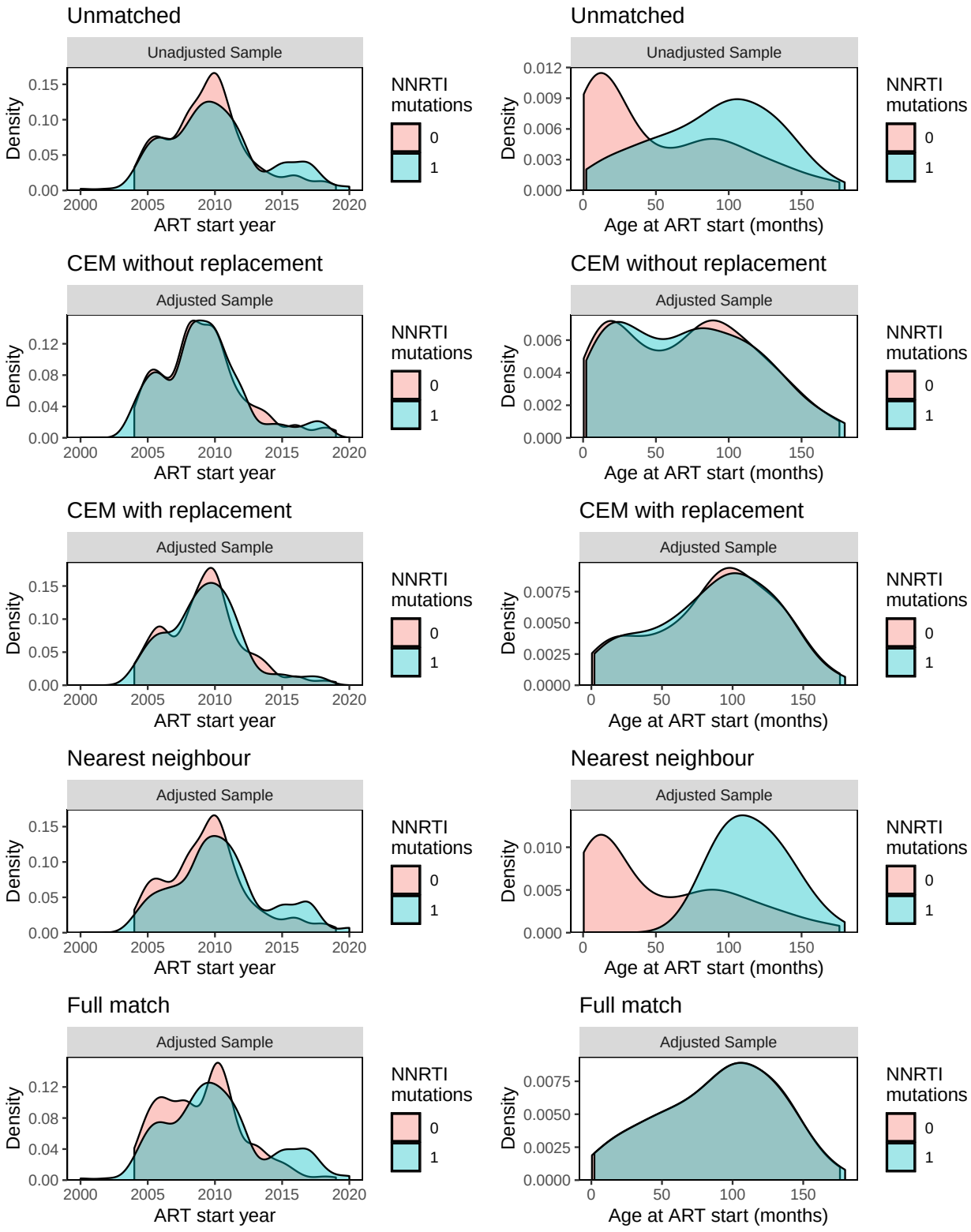

Figure 2.4: Distributional balance for ART start age and ART start age for NNRTI resistance mutation analysis

CEM = coarsened exact matching; NNRTI = non-nucleoside reverse transcriptase inhibitor

Table 2.9: Multivariable Logistic Regression Outputs comparing cases with and without NNRTI resistance mutations: Unmatched and Matched Case-Control Models

Parameter	Adjusted Odds Ratio	Lower Est	Upper Est	P-value
Unmatched				
Intercept	NE	NE	NE	-
ART start age	1.02	1.014	1.026	<0.001
Early Middle (Yes)	NE	NE	NE	-
Late Middle (Yes)	NE	NE	NE	-
Late (Yes)	NE	NE	NE	-
TB pre-resistance test (Yes)	0.863	0.529	1.413	0.557
Orphaned pre-resistance test (Yes)	0.927	0.553	1.538	0.772
Received PMTCT (Yes)	0.716	0.353	1.44	0.35
Received PMTCT (Unknown)	1.758	1.021	3.081	0.045
Ever SAM (Yes)	1.54	0.866	2.783	0.146
Coarsened Exact Matching without replacement				
Intercept	0.81	0.442	1.486	0.497
ART start age	1.001	0.997	1.005	0.675
Early Middle (Yes)	1.13	0.673	1.897	0.644
Late Middle (Yes)	1.27	0.93	1.734	0.132
TB pre-resistance test (Yes)	0.983	0.532	1.817	0.958
Orphaned pre-resistance test (Yes)	1.201	0.663	2.176	0.546
Received PMTCT (Yes)	0.678	0.268	1.716	0.412
Received PMTCT (Unknown)	1.2	0.602	2.391	0.604
Ever SAM (Yes)	1.737	0.788	3.829	0.171
Coarsened Exact Matching with replacement				
Intercept	0.861	0.315	2.355	0.771
ART start age	1.003	0.995	1.012	0.455
Early Middle (Yes)	0.994	0.356	2.773	0.991
Late Middle (Yes)	1.256	0.599	2.633	0.547
TB pre-resistance test (Yes)	1.285	0.661	2.498	0.461
Orphaned pre-resistance test (Yes)	0.746	0.367	1.517	0.418
Received PMTCT (Yes)	1.016	0.408	2.531	0.973
Received PMTCT (Unknown)	1.695	0.751	3.826	0.204

Table 2.9: Multivariable Logistic Regression Outputs comparing cases with and without NNRTI resistance mutations: Unmatched and Matched Case-Control Models *(continued)*

Parameter	Adjusted Odds Ratio	Lower Est	Upper Est	P-value
Ever SAM (Yes)	1.932	0.857	4.353	0.112
Nearest Neighbour Matching				
Intercept	0.021	0.007	0.066	<0.001
ART start age	1.044	1.033	1.055	<0.001
Early Middle (Yes)	1.181	0.533	2.615	0.681
Late Middle (Yes)	1.6	0.874	2.93	0.128
TB pre-resistance test (Yes)	0.985	0.49	1.978	0.965
Orphaned pre-resistance test (Yes)	0.881	0.463	1.678	0.7
Received PMTCT (Yes)	1.657	0.691	3.976	0.258
Received PMTCT (Unknown)	1.643	0.789	3.423	0.185
Ever SAM (Yes)	1.216	0.516	2.866	0.654
Full Matching				
Intercept	53.614	26.807	107.228	<0.001
ART start age	1.003	0.999	1.008	0.148
Early Middle (Yes)	NE	NE	0	-
Late Middle (Yes)	2933.029	1128.834	7620.836	<0.001
Late (Yes)	0.061	0.034	0.108	<0.001
TB pre-resistance test (Yes)	1.063	0.562	2.008	0.851
Orphaned pre-resistance test (Yes)	0.698	0.355	1.37	0.296
Received PMTCT (Yes)	1.455	0.526	4.029	0.47
Received PMTCT (Unknown)	1.462	0.696	3.074	0.316
Ever SAM (Yes)	1.882	0.787	4.502	0.155

Table 2.10: Matching for case-control analysis of risk factors associated with PI mutations in children and adolescents that underwent ART resistance testing

	Means Cases	Means Controls	Std Mean Diff	Var Ratio	eCDF Mean	eCDF Distance	Pair Dist
Unmatched: 19 Cases, 406 Controls							
Distance	0.1	0.0	1.0	1.2	0.3	0.5	-
ART Year	2010.5	2009.8	0.2	0.8	0.1	0.1	-
ART Age	31.8	77.3	-1.1	0.7	0.3	0.5	-
Coarsened Exact Matching without replacement: 19 Cases, 19 Controls							
ART Year	2010.5	2010.5	0.0	1.0	0.0	0.1	0
ART Age	31.8	30.7	0.0	1.0	0.0	0.2	0.1
Coarsened Exact Matching with replacement: 19 Cases, 94 Controls							
ART Year	2010.5	2010.6	0.0	1.0	0.0	0.1	0.1
ART Age	31.8	31.7	0.0	1.0	0.0	0.2	0.1
Nearest Neighbour Matching: 19 Cases, 19 Controls							
Distance	0.1	0.1	0.0	1.0	0.0	0.1	0
ART Year	2010.5	2009.1	0.5	2.1	0.1	0.3	0.9
ART Age	31.8	31.8	0.0	1.0	0.0	0.1	0
Full Matching: 19 Cases, 406 Controls							
Distance	0.1	0.1	0.0	1.0	0.0	0.1	0.1
ART Year	2010.5	2009.7	0.3	1.0	0.0	0.1	1.2
ART Age	31.8	31.8	0.0	1.1	0.0	0.1	0.2

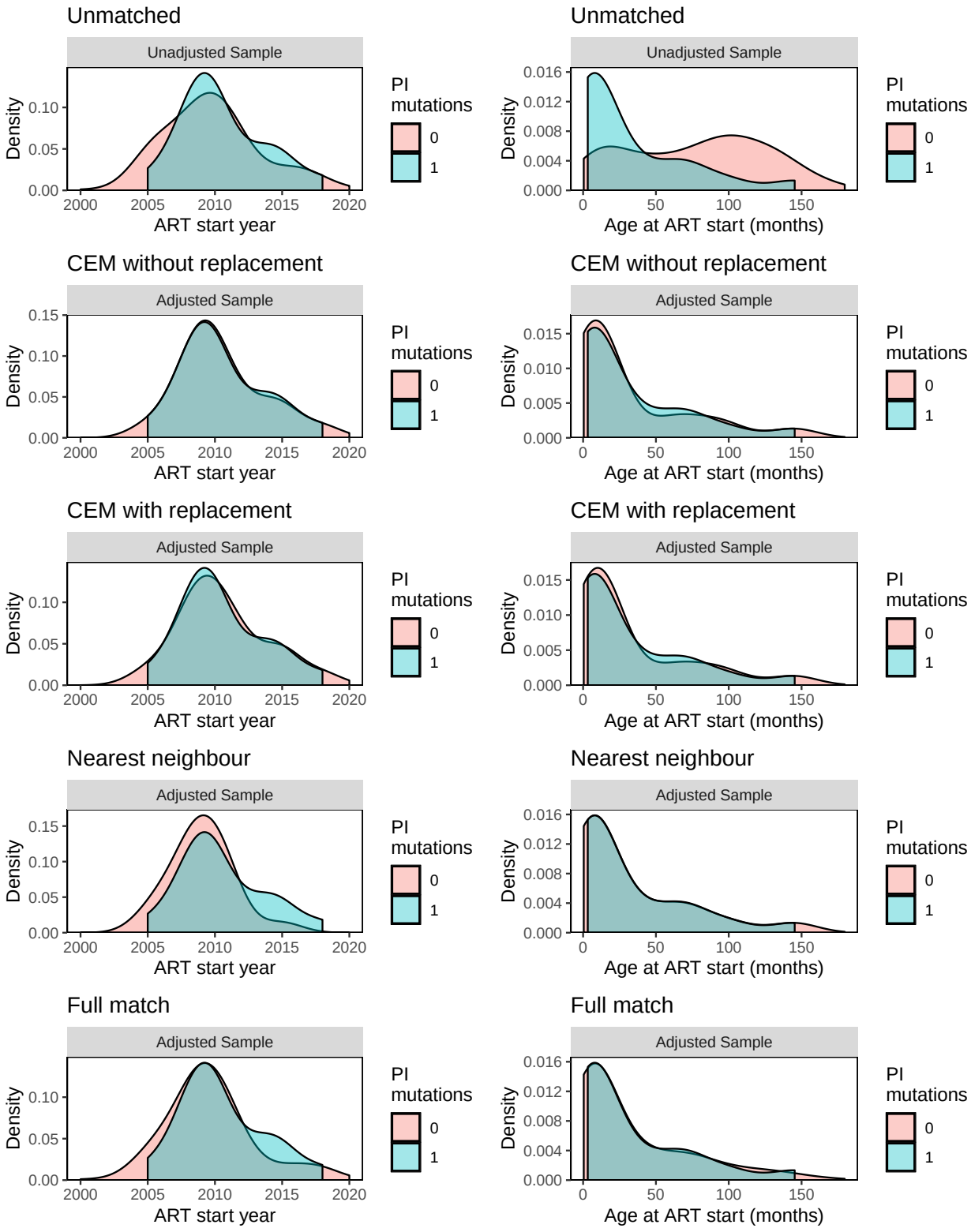

Figure 2.5: Distributional balance for ART start age and ART start age for PI resistance mutation analysis

CEM = coarsened exact matching; PI = protease inhibitor

Table 2.11: Multivariable Logistic Regression Outputs comparing cases with and without PI resistance mutations: Unmatched and Matched Case-Control Models

Parameter	Adjusted Odds Ratio	Lower Est	Upper Est	P-value
Unmatched				
Intercept	NE	NE	NE	-
ART start age	0.975	0.959	0.989	0.001
Early Middle (Yes)	NE	NE	NE	-
Late Middle (Yes)	NE	NE	NE	-
Late (Yes)	NE	NE	NE	-
TB pre-resistance test (Yes)	1.391	0.482	3.872	0.53
Orphaned pre-resistance test (Yes)	1.975	0.624	6.507	0.25
Received PMTCT (Yes)	0.741	0.17	2.88	0.67
Received PMTCT (Unknown)	1.333	0.431	4.159	0.614
Ever SAM (Yes)	2.37	0.828	6.731	0.102
Coarsened Exact Matching without replacement				
Intercept	0.402	0.078	2.081	0.278
ART start age	1	0.981	1.019	0.981
Early Middle (Yes)	1.651	0.374	7.285	0.508
Late Middle (Yes)	1.119	0.418	2.993	0.823
TB pre-resistance test (Yes)	1.811	0.364	9.017	0.468
Orphaned pre-resistance test (Yes)	1.799	0.264	12.252	0.549
Received PMTCT (Yes)	1.071	0.259	4.425	0.925
Received PMTCT (Unknown)	3.576	0.594	21.519	0.164
Ever SAM (Yes)	2.227	0.359	13.809	0.39
Coarsened Exact Matching with replacement				
Intercept	0.082	0.011	0.585	0.013
ART start age	1.004	0.979	1.031	0.738
Early Middle (Yes)	1.404	0.213	9.235	0.724
Late Middle (Yes)	0.721	0.177	2.939	0.649
TB pre-resistance test (Yes)	1.415	0.396	5.055	0.593
Orphaned pre-resistance test (Yes)	2.28	0.488	10.645	0.294
Received PMTCT (Yes)	0.821	0.128	5.263	0.835
Received PMTCT (Unknown)	1.415	0.306	6.542	0.657

Table 2.11: Multivariable Logistic Regression Outputs comparing cases with and without PI resistance mutations: Unmatched and Matched Case-Control Models *(continued)*

Parameter	Adjusted Odds Ratio	Lower Est	Upper Est	P-value
Ever SAM (Yes)	2.689	0.718	10.069	0.142
Nearest Neighbour Matching				
Intercept	0.635	0.109	3.707	0.614
ART start age	1	0.987	1.014	0.961
Early Middle (Yes)	3.09	0.584	16.348	0.184
Late Middle (Yes)	1.112	0.244	5.065	0.891
TB pre-resistance test (Yes)	1.125	0.168	7.556	0.903
Orphaned pre-resistance test (Yes)	2.029	0.368	11.2	0.417
Received PMTCT (Yes)	0.803	0.121	5.334	0.82
Received PMTCT (Unknown)	1.686	0.263	10.818	0.582
Ever SAM (Yes)	2.602	0.618	10.959	0.192
Full Matching				
Intercept	NE	NE	0.005	-
ART start age	0.999	0.992	1.006	0.785
Early Middle (Yes)	9178.077	1810.79	NE	<0.001
Late Middle (Yes)	0.004	0.001	0.023	<0.001
Late (Yes)	16.151	7.289	35.786	<0.001
TB pre-resistance test (Yes)	1.452	0.455	4.63	0.529
Orphaned pre-resistance test (Yes)	1.644	0.339	7.966	0.537
Received PMTCT (Yes)	0.545	0.084	3.545	0.525
Received PMTCT (Unknown)	1.052	0.322	3.432	0.933
Ever SAM (Yes)	3.183	0.683	14.829	0.14

Table 2.12: Case-Control matching for analyses exploring factors associated with resistance testing in children and adolescents attending the ART clinic during the study period

	Means Cases	Means Controls	Std Mean Diff	Var Ratio	eCDF Mean	eCDF Distance	Pair Dist
Unmatched: Cases, 425 Controls							
Distance	0.3	0.3	0.4	1.0	0.1	0.2	-
ART Year	2009.8	2007.8	0.6	1.2	0.1	0.3	-
ART Age	75.3	57.8	0.4	0.9	0.1	0.2	-
Coarsened Exact Matching without replacement: 305 Cases, 305 Controls							
ART Year	2008.8	2008.9	0.0	1.0	0.0	0.0	0
ART Age	66.0	65.8	0.0	1.0	0.0	0.0	0
Coarsened Exact Matching with replacement: 392 Cases, 867 Controls							
ART Year	2009.4	2009.4	0.0	1.0	0.0	0.0	0.1
ART Age	72.4	72.0	0.0	1.0	0.0	0.0	0.1
Nearest Neighbour Matching: 425 Cases, 425 Controls							
Distance	0.3	0.3	0.0	1.0	0.0	0.0	0
ART Year	2009.8	2007.8	0.6	1.1	0.1	0.3	1.1
ART Age	75.3	74.9	0.0	1.0	0.0	0.0	0
Full Matching: 425 Cases, 953 Controls							
Distance	0.3	0.3	0.0	1.0	0.0	0.0	0
ART Year	2009.8	2007.7	0.6	1.2	0.1	0.4	1.2
ART Age	75.3	75.4	0.0	1.0	0.0	0.0	0

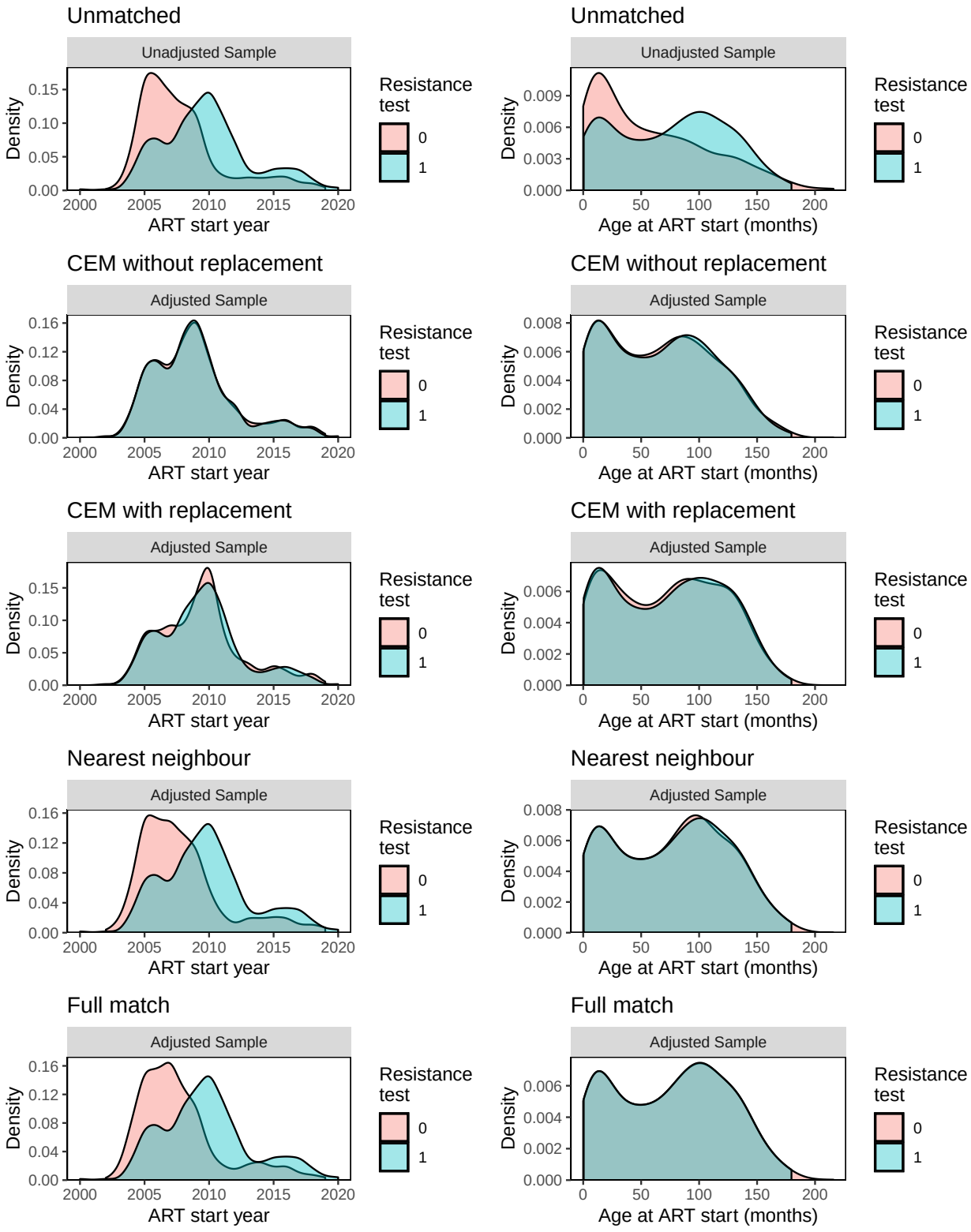

Figure 2.6: Distributional balance for ART start age and ART start age in each of the case-control data sets

CEM = coarsened exact matching

Table 2.13: Matched case-control data using coarsened exact matching (nearest neighbour matching without replacement within each stratum)

	Overall	Controls	Cases	p	test
n	610	305	305		
Male (%)	319 (52.3)	156 (51.1)	163 (53.4)	0.627	
Median age [months, IQR] at ART initiation	65.68 [21.79, 101.42]	65.37 [22.55, 101.40]	67.48 [21.72, 101.92]	0.908	nonnorm
Median CD4 percentage [%, IQR] at baseline	15.75 [8.86, 24.00]	17.40 [9.58, 25.09]	14.05 [8.41, 21.08]	0.002	nonnorm
Median CD4 count [cells/uL, IQR] at baseline	466.00 [222.00, 890.00]	476.00 [278.00, 896.00]	432.00 [195.75, 838.00]	0.106	nonnorm
Baseline immunologic status (%)				0.010	
None	121 (19.9)	67 (22.0)	54 (17.8)		
Mild	83 (13.6)	52 (17.0)	31 (10.2)		
Advanced	69 (11.3)	37 (12.1)	32 (10.5)		
Severe	336 (55.2)	149 (48.9)	187 (61.5)		
Median HIVVL [log10 RNA copies/mL, IQR] at baseline	4.80 [3.89, 5.57]	4.75 [3.91, 5.51]	4.86 [3.87, 5.59]	0.779	nonnorm
ART era (%)				0.998	
Early (<2004)	2 (0.3)	1 (0.3)	1 (0.3)		
Early Middle (2004 to end-2009)	399 (66.2)	203 (66.6)	196 (65.8)		
Late Middle (2010 to end-2014)	152 (25.2)	76 (24.9)	76 (25.5)		
Late (2015 to end-2020)	50 (8.3)	25 (8.2)	25 (8.4)		
Ever diagnosed with tuberculosis (%)	189 (31.0)	82 (26.9)	107 (35.1)	0.036	
Tuberculosis diagnosed prior to resistance test (%)	175 (28.7)	82 (26.9)	93 (30.5)	0.371	
Ever orphaned (%)	356 (58.4)	166 (54.4)	190 (62.3)	0.059	
Orphaned prior to resistance test (%)	338 (55.4)	166 (54.4)	172 (56.4)	0.684	
PMTCT (%)				0.733	
No	362 (59.3)	185 (60.7)	177 (58.0)		
Yes	104 (17.0)	52 (17.0)	52 (17.0)		
Unknown	144 (23.6)	68 (22.3)	76 (24.9)		
Ever diagnosed with SAM (%)	77 (16.4)	15 (8.7)	62 (20.9)	0.001	

Note:
ART = antiretroviral therapy; HIVVL = HIV viral load in whole blood; IQR = interquartile range; PMTCT = prevention of mother-to-child transmission; SAM = severe acute malnutrition; TB = tuberculosis. P-values are derived from comparison of groups: Chi-square test or Fisher's exact test, as appropriate, for categorical variables; Student t test for comparison of means; Kruskal-Wallis test for comparison of medians.

Table 2.14: Univariate outputs for coarsened exact matched case-control data (without replacement)

Parameter	Odds Ratio	Lower Est	Upper Est	P-value
Male (Yes)	1.1	0.8	1.51	0.575
Age at ART start (months)	1.00	1.00	1.00	0.238
ART start year	1.00	1.00	1.00	0.466
ART era: Early Middle	1.01	1.00	1.01	0.008
ART era: Late Middle (Yes)	1.02	1.00	1.03	0.008
ART era: Late (Yes)	0.98	0.96	0.99	0.008
Ever SAM (Yes)	2.76	1.59	4.8	<0.001
Baseline immunologic status: Mild	1.47	1.05	2.05	0.023
Baseline immunologic status: Advanced	1.38	1.02	1.87	0.035
Baseline immunologic status: Severe	0.74	0.44	1.25	0.263
Ever TB (Yes)	1.07	0.6	1.92	0.812
Ever orphaned (Yes)	1.56	1.1	2.2	0.013
Baseline CD4%	0.98	0.96	0.99	0.001
Baseline CD4 count	1.00	1.00	1.00	0.372
Baseline log10 HIVVL	1.00	0.89	1.11	0.95
TB pre-resistance test (Yes)	1.19	0.85	1.67	0.301
Orphaned pre-resistance test (Yes)	1.08	0.8	1.47	0.607
PMTCT (Yes)	1.05	0.74	1.47	0.799
PMTCT (Unknown)	1.17	0.84	1.63	0.358

Table 2.15: Matched case-control data using coarsened exact matching (nearest neighbour matching with replacement within each stratum)

	Overall	Controls	Cases	p	test
n	1259	867	392		
Male (%)	646 (51.3)	440 (50.7)	206 (52.6)	0.595	
Median age [months, IQR] at ART initiation	48.91 [14.68, 92.92]	39.41 [12.28, 81.47]	76.84 [24.01, 112.39]	<0.001	nonnorm
Median CD4 percentage [%, IQR] at baseline	16.00 [9.68, 23.67]	17.02 [10.95, 24.15]	13.73 [7.37, 20.40]	<0.001	nonnorm
Median CD4 count [cells/uL, IQR] at baseline	541.00 [263.25, 1000.50]	606.00 [329.00, 1073.00]	379.00 [168.50, 810.50]	<0.001	nonnorm
Baseline immunologic status (%)				0.014	
None	231 (18.4)	166 (19.1)	65 (16.6)		
Mild	162 (12.9)	123 (14.2)	39 (10.0)		
Advanced	149 (11.8)	110 (12.7)	39 (10.0)		
Severe	716 (56.9)	468 (54.0)	248 (63.4)		
Median HIVVL [log10 RNA copies/mL, IQR] at baseline	4.95 [3.98, 5.69]	4.96 [4.00, 5.75]	4.90 [3.92, 5.60]	0.391	nonnorm
ART era (%)				<0.001	
Early (<2004)	2 (0.2)	1 (0.1)	1 (0.3)		
Early Middle (2004 to end-2009)	901 (72.0)	705 (81.3)	196 (50.9)		
Late Middle (2010 to end-2014)	251 (20.0)	99 (11.4)	152 (39.5)		
Late (2015 to end-2020)	98 (7.8)	62 (7.2)	36 (9.4)		
Ever diagnosed with tuberculosis (%)	379 (30.1)	247 (28.5)	132 (33.7)	0.073	
Tuberculosis diagnosed prior to resistance test (%)	362 (28.8)	247 (28.5)	115 (29.3)	0.810	
Ever orphaned (%)	668 (53.1)	430 (49.6)	238 (60.7)	<0.001	
Orphaned prior to resistance test (%)	647 (51.4)	430 (49.6)	217 (55.4)	0.067	
PMTCT (%)				0.427	
No	718 (57.0)	492 (56.7)	226 (57.7)		
Yes	224 (17.8)	162 (18.7)	62 (15.8)		
Unknown	317 (25.2)	213 (24.6)	104 (26.5)		
Ever diagnosed with SAM (%)	148 (15.0)	71 (11.7)	77 (20.2)	<0.001	

Note:
ART = antiretroviral therapy; HIVVL = HIV viral load in whole blood; IQR = interquartile range; PMTCT = prevention of mother-to-child transmission; SAM = severe acute malnutrition; TB = tuberculosis. P-values are derived from comparison of groups: Chi-square test or Fisher's exact test, as appropriate, for categorical variables; Student t test for comparison of means; Kruskal-Wallis test for comparison of medians.

Table 2.16: Univariate outputs for coarsened exact matched case-control data (with replacement)

Parameter	Odds Ratio	Lower Est	Upper Est	P-value
Male (Yes)	1.02	0.74	1.39	0.919
Age at ART start (months)	1.00	1.00	1.00	0.927
ART start year	1.01	0.96	1.06	0.783
ART era: Early Middle	1.01	0.01	105.73	0.997
ART era: Late Middle (Yes)	1.02	0.03	32.78	0.992
ART era: Late (Yes)	0.98	0.2	4.69	0.977
Ever SAM (Yes)	3.37	2.15	5.28	<0.001
Baseline immunologic status: Mild	1.38	0.99	1.94	0.061
Baseline immunologic status: Advanced	1.26	0.92	1.73	0.156
Baseline immunologic status: Severe	1.12	0.65	1.94	0.683
Ever TB (Yes)	1.32	0.74	2.36	0.351
Ever orphaned (Yes)	2.21	1.45	3.35	<0.001
Baseline CD4%	0.96	0.95	0.98	<0.001
Baseline CD4 count	1.00	1.00	1.00	0.073
Baseline log10 HIVVL	1.09	0.97	1.22	0.13
TB pre-resistance test (Yes)	1.13	0.8	1.6	0.483
Orphaned pre-resistance test (Yes)	1.01	0.74	1.38	0.951
PMTCT (Yes)	1.15	0.76	1.72	0.511
PMTCT (Unknown)	1.18	0.8	1.74	0.396

Table 2.17: Matched case-control data using nearest neighbour matching

	Overall	Controls	Cases	p	test
n	850	425	425		
Male (%)	427 (50.2)	205 (48.2)	222 (52.2)	0.272	
Median age [months, IQR] at ART initiation	81.51 [28.42, 112.52]	81.43 [28.43, 110.85]	81.61 [28.42, 112.78]	0.893	nonnorm
Median CD4 percentage [%, IQR] at baseline	15.40 [8.42, 22.50]	16.80 [10.20, 23.60]	13.77 [7.05, 20.44]	<0.001	nonnorm
Median CD4 count [cells/uL, IQR] at baseline	435.00 [190.00, 794.00]	481.00 [238.00, 804.00]	381.00 [162.00, 762.75]	0.017	nonnorm
Baseline immunologic status (%)				0.012	
None	173 (20.4)	98 (23.1)	75 (17.7)		
Mild	100 (11.8)	57 (13.4)	43 (10.1)		
Advanced	95 (11.2)	53 (12.5)	42 (9.9)		
Severe	481 (56.7)	217 (51.1)	264 (62.3)		
Median HIVVL [log10 RNA copies/mL, IQR] at baseline	4.82 [3.89, 5.56]	4.75 [3.81, 5.48]	4.89 [3.91, 5.58]	0.221	nonnorm
ART era (%)				<0.001	
Early (<2004)	9 (1.1)	7 (1.6)	2 (0.5)		
Early Middle (2004 to end-2009)	530 (62.9)	334 (78.6)	196 (46.9)		
Late Middle (2010 to end-2014)	221 (26.2)	54 (12.7)	167 (40.0)		
Late (2015 to end-2020)	83 (9.8)	30 (7.1)	53 (12.7)		
Ever diagnosed with tuberculosis (%)	240 (28.2)	99 (23.3)	141 (33.2)	0.002	
Tuberculosis diagnosed prior to resistance test (%)	222 (26.1)	99 (23.3)	123 (28.9)	0.073	
Ever orphaned (%)	477 (56.1)	232 (54.6)	245 (57.6)	0.407	
Orphaned prior to resistance test (%)	455 (53.5)	232 (54.6)	223 (52.5)	0.582	
PMTCT (%)				<0.001	
No	536 (63.1)	297 (69.9)	239 (56.2)		
Yes	112 (13.2)	49 (11.5)	63 (14.8)		
Unknown	202 (23.8)	79 (18.6)	123 (28.9)		
Ever diagnosed with SAM (%)	96 (15.5)	14 (6.9)	82 (19.8)	<0.001	

Note:
ART = antiretroviral therapy; HIVVL = HIV viral load in whole blood; IQR = interquartile range; PMTCT = prevention of mother-to-child transmission; SAM = severe acute malnutrition; TB = tuberculosis. P-values are derived from comparison of groups: Chi-square test or Fisher's exact test, as appropriate, for categorical variables; Student t test for comparison of means; Kruskal-Wallis test for comparison of medians.

Table 2.18: Univariate outputs for nearest neighbour matched case-control data

Parameter	Odds Ratio	Lower Est	Upper Est	P-value
Male (Yes)	1.17	0.9	1.53	0.235
Age at ART start (months)	1.00	1.00	1.00	<0.001
ART start year	1.19	1.14	1.25	<0.001
ART era: Early Middle	4.92	1.63	14.9	0.005
ART era: Late Middle (Yes)	0.53	0.23	1.21	0.131
ART era: Late (Yes)	0.49	0.32	0.76	0.002
Ever SAM (Yes)	3.35	1.9	5.92	<0.001
Baseline immunologic status: Mild	1.63	1.21	2.2	0.001
Baseline immunologic status: Advanced	1.13	0.87	1.47	0.35
Baseline immunologic status: Severe	0.99	0.61	1.6	0.954
Ever TB (Yes)	1.04	0.63	1.72	0.892
Ever orphaned (Yes)	1.59	1.14	2.22	0.006
Baseline CD4%	0.98	0.96	0.99	<0.001
Baseline CD4 count	1.00	1.00	1.00	0.483
Baseline log10 HIVVL	1.05	0.96	1.15	0.274
TB pre-resistance test (Yes)	1.34	0.99	1.81	0.056
Orphaned pre-resistance test (Yes)	0.92	0.71	1.19	0.526
PMTCT (Yes)	1.6	1.1	2.31	0.013
PMTCT (Unknown)	1.93	1.39	2.69	<0.001

Table 2.19: Matched case-control data using full matching

	Overall	Controls	Cases	p	test
n	1378	953	425		
Male (%)	693 (50.3)	471 (49.4)	222 (52.2)	0.365	
Median age [months, IQR] at ART initiation	55.82 [16.34, 101.10]	45.18 [13.30, 91.81]	81.61 [28.42, 112.78]	<0.001	nonnorm
Median CD4 percentage [%, IQR] at baseline	15.90 [9.44, 23.40]	16.63 [10.57, 24.00]	13.77 [7.05, 20.44]	<0.001	nonnorm
Median CD4 count [cells/uL, IQR] at baseline	517.00 [242.00, 948.00]	566.00 [294.00, 1019.00]	381.00 [162.00, 762.75]	<0.001	nonnorm
Baseline immunologic status (%)				0.039	
None	251 (18.2)	176 (18.5)	75 (17.7)		
Mild	176 (12.8)	133 (14.0)	43 (10.1)		
Advanced	164 (11.9)	122 (12.8)	42 (9.9)		
Severe	786 (57.1)	522 (54.8)	264 (62.3)		
Median HIVVL [log10 RNA copies/mL, IQR] at baseline	4.91 [3.93, 5.65]	4.93 [3.94, 5.70]	4.89 [3.91, 5.58]	0.523	nonnorm
ART era (%)				<0.001	
Early (<2004)	10 (0.7)	8 (0.8)	2 (0.5)		
Early Middle (2004 to end-2009)	968 (70.6)	772 (81.0)	196 (46.9)		
Late Middle (2010 to end-2014)	275 (20.1)	108 (11.3)	167 (40.0)		
Late (2015 to end-2020)	118 (8.6)	65 (6.8)	53 (12.7)		
Ever diagnosed with tuberculosis (%)	402 (29.2)	261 (27.4)	141 (33.2)	0.034	
Tuberculosis diagnosed prior to resistance test (%)	384 (27.9)	261 (27.4)	123 (28.9)	0.597	
Ever orphaned (%)	727 (52.8)	482 (50.6)	245 (57.6)	0.018	
Orphaned prior to resistance test (%)	705 (51.2)	482 (50.6)	223 (52.5)	0.554	
PMTCT (%)				0.061	
No	801 (58.1)	562 (59.0)	239 (56.2)		
Yes	232 (16.8)	169 (17.7)	63 (14.8)		
Unknown	345 (25.0)	222 (23.3)	123 (28.9)		
Ever diagnosed with SAM (%)	155 (15.0)	73 (11.7)	82 (19.8)	0.001	

Note:
ART = antiretroviral therapy; HIVVL = HIV viral load in whole blood; IQR = interquartile range; PMTCT = prevention of mother-to-child transmission; SAM = severe acute malnutrition. P-values are derived from comparison of groups: Chi-square test or Fisher's exact test, as appropriate, for categorical variables; Student t test for comparison of means; Kruskal-Wallis test for comparison of medians.

Table 2.20: Univariate outputs for full matching of case-control data

Parameter	Odds Ratio	Lower Est	Upper Est	P-value
Male (Yes)	1.18	0.9	1.54	0.222
Age at ART start (months)	1.00	1.00	1.00	0.001
ART start year	1.19	1.14	1.25	<0.001
ART era: Early Middle	5.36	1.73	16.64	0.004
ART era: Late Middle (Yes)	0.59	0.25	1.41	0.239
ART era: Late (Yes)	0.48	0.31	0.76	0.002
Ever SAM (Yes)	2.56	1.7	3.86	<0.001
Baseline immunologic status: Mild	1.56	1.15	2.12	0.004
Baseline immunologic status: Advanced	1.14	0.87	1.49	0.334
Baseline immunologic status: Severe	0.88	0.54	1.42	0.594
Ever TB (Yes)	0.91	0.54	1.55	0.739
Ever orphaned (Yes)	1.61	1.11	2.33	0.012
Baseline CD4%	0.98	0.96	0.99	0.002
Baseline CD4 count	1.00	1.00	1.00	0.261
Baseline log10 HIVVL	1.09	1.00	1.2	0.059
TB pre-resistance test (Yes)	1.28	0.94	1.75	0.121
Orphaned pre-resistance test (Yes)	0.93	0.7	1.22	0.578
PMTCT (Yes)	1.46	1.01	2.1	0.045
PMTCT (Unknown)	2.11	1.55	2.88	<0.001

Table 2.21: Characteristics of children with 3 or more PI mutations, compared to those with 1 or 2 PI mutations

	Overall	<3 PI mutations	3 to 5 PI mutations	p
n	26	12	14	
Male (%)	18 (69.2)	9 (75.0)	9 (64.3)	0.870
Median age at ART start (months) [IQR]	16.31 [5.37, 52.32]	33.17 [14.28, 56.70]	6.98 [4.98, 35.65]	0.258
Mean baseline CD4 percentage (SD)	16.12 (9.11)	17.11 (11.49)	15.26 (6.79)	0.615
Mean baseline CD4 count (SD)	680.50 (468.75)	743.92 (425.00)	626.14 (512.66)	0.534
Baseline immunologic status (%)				0.276
None	3 (11.5)	2 (16.7)	1 (7.1)	
Mild	6 (23.1)	3 (25.0)	3 (21.4)	
Advanced	2 (7.7)	2 (16.7)	0 (0.0)	
Severe	15 (57.7)	5 (41.7)	10 (71.4)	
Mean baseline HIVVL (log10 copies/mL) (SD)	4.82 (1.62)	4.55 (1.85)	5.05 (1.43)	0.442
ART Era (%)				0.738
Early Middle (2004 to end-2009)	12 (46.2)	5 (41.7)	7 (50.0)	
Late Middle (2010 to end-2014)	11 (42.3)	5 (41.7)	6 (42.9)	
Late (2015 to end-2020)	3 (11.5)	2 (16.7)	1 (7.1)	
Ever TB (%)	11 (42.3)	5 (41.7)	6 (42.9)	1.000
TB pre-resistance test (%)	9 (34.6)	4 (33.3)	5 (35.7)	1.000
Ever orphaned (%)	14 (53.8)	7 (58.3)	7 (50.0)	0.976
Orphaned pre-resistance test (%)	13 (50.0)	7 (58.3)	6 (42.9)	0.694
PMTCT (%)				0.042
Not received	12 (46.2)	8 (66.7)	4 (28.6)	
Received	5 (19.2)	0 (0.0)	5 (35.7)	
Unknown	9 (34.6)	4 (33.3)	5 (35.7)	
Ever SAM (Yes, %)	11 (42.3)	4 (33.3)	7 (50.0)	0.646

Note:
ART = antiretroviral therapy; BMI = body mass index; HFA = height-for-age; HIVVL = HIV viral load in whole blood; IQR = interquartile range; NNRTI = non-nucleoside reverse transcriptase inhibitor; PI = protease inhibitor; SD = standard deviation; WFA = weight-for-age; WFH = weight-for-height. P-values are derived from comparison of groups: Chi-square test or Fisher's exact test, as appropriate, for categorical variables; Student t test for comparison of means; Kruskal-Wallis test for comparison of medians.

APPENDIX 2: Study Protocol

PATTERNS OF HIV RESISTANCE IN CHILDREN ATTENDING AN ANTIRETROVIRAL CLINIC IN SOWETO, SOUTH AFRICA

A case-control study

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ABBREVIATIONS

3TC:	Lamivudine
ADR:	Acquired drug resistance
AIDS:	Acquired immunodeficiency syndrome
ART:	Antiretroviral therapy
ARV:	Antiretroviral
CHBAH:	Chris Hani Baragwanath Academic Hospital
CLWH:	Children living with HIV
DRMs:	Drug resistance mutations
EFV:	Efavirenz
FTC:	Emtricitabine
HIV:	Human immunodeficiency virus
HSCC:	Harriet Shezi Children's Clinic
IQR:	Interquartile range
NHLS:	National Health Laboratory Service
NNRTI:	Non-nucleoside reverse transcriptase inhibitors
NRTI:	Nucleotide reverse transcriptase inhibitors
NVP:	Nevirapine
PDR:	Pre-treatment drug resistance
PI:	Protease inhibitor
PLWH:	Person/people living with HIV
PMTCT:	Prevention of mother-to-child transmission
SD:	Standard deviation
TDR:	Transmitted drug resistance
VL:	Viral load
WHO:	World Health Organisation

INTRODUCTION

Human immunodeficiency virus (HIV) infection remains a major global epidemic. UNICEF estimates that of the 37.9 million people living with HIV (PLWH) worldwide at the end of 2018, 2.8 million of them were children between the ages of 0-19 (1). In South Africa it is estimated that 7.7 million people were living with HIV in 2018 (2), with half a million of them being children between the ages of 0-19 years (3). South Africa currently has the largest antiretroviral therapy (ART) treatment programme in the world since its introduction in 2004, with an estimated 3.1 million people receiving ART (4). The discovery and roll out of ART has improved the quality of life and life expectancy of PLWH substantially. The goals of ART are to inhibit viral replication, halt the progression of disease to acquired immunodeficiency syndrome (AIDS), and to allow for partial restoration of the immune system (5).

ART and emergence of mutations

Exposure to antimicrobials will inevitably lead to antimicrobial resistance, and exposure to ART has also led to HIV resistance (6). When HIV replicates in the presence of ART, genomic mutations occur which alter the viral proteins that are targeted by antiretroviral (ARV) drugs, which leads to different types of mutations, some of which may lead to treatment failure (5). The World Health Organisation (WHO) defines ARV drug resistance as mutations in the genetic structure of HIV that affects the ability of a specific drug or combination of drugs to block replication of HIV (7). All current ARV drugs, including newer classes, are at risk of becoming partly or fully inactive because of the emergence of drug-resistant strains (8).

HIV is particularly prone to developing resistance for a number of reasons, including high levels of circulating virus and rapid and error prone reverse transcription of the genome. The process of reverse transcription is inaccurate, due to the absence of any enzymatic

proofreading activity (5). This leads to each genome having at least one mutation which results in diverse HIV populations, some of which have a survival advantage especially when they make the virus less susceptible to some of the ARV drugs (5).

Classification of drug resistance

HIV drug resistance is classified into three categories by the WHO: 1) acquired HIV drug resistance (ARD); 2) transmitted HIV drug resistance (TDR); and 3) pre-treatment HIV drug resistance (PDR). ARD (acquired resistance) is the most common type of drug resistance and occurs when HIV replicates in the presence of ART, where drug levels are too low to block replication but high enough to exert a positive selection pressure on the virus (5)(7). TDR (transmitted resistance) is detected among ARV drug naïve people with no history of ARV drug exposure, and occurs through infection with virus that has drug resistance mutations (DRMs) (8). PDR (pre-treatment resistance) refers to resistance that is detected among ARV naïve people initiating ART, or people with previous ARV drug exposure initiating or reinitiating first-line ART (8). PDR is clinically important as it is associated with a poor response to first-line therapy and further accumulation of drug resistance mutations (9).

The WHO reports that in several low and middle income countries, more than 1 in 10 adults starting ART were infected with HIV that was already resistant to efavirenz (EFV) or nevirapine (NVP). In these groups, women were twice as likely as men to carry a PDR virus. This challenges the elimination of mother-to-child transmission of HIV, and impacts on maternal and child health outcomes (7). Furthermore, 25% persons initiating first-line ART had previous ARV exposure, either in the form of prevention-of-mother-to-child transmission (PMTCT) or treatment interruption, and were three times more likely to have PDR to EFV or NVP compared to those with no prior antiretroviral exposure (7).

HIV mutations in children

Children present with more HIV drug resistance than adults, and have a 2-fold higher risk of virological failure (VF) compared to adults after 5 years on ART (10). In surveys conducted by the WHO in nine sub-Saharan African countries between 2012 and 2018, the prevalence of PDR among children under 18 months with newly diagnosed HIV was high: over half of the infants carried a virus that was resistant to EFV and/or NVP (8). The prevalence of PDR to nucleoside reserve-transcriptase inhibitors (NRTIs) also exceeded 10% in newly diagnosed infants in some countries included in the surveys (8).

A multicounty analysis performed between 2011-2014, reported one in two children under the age of 18 months, who are newly diagnosed with HIV are infected with a strain that is resistant to non-nucleoside reverse transcriptase inhibitors (NNRTIs), and are therefore at high risk for suboptimal virological response to treatment if initiated on an NNRTI-based regimen (11). Risk factors for children with multiclass ARV resistance included PMTCT exposure, sub-optimal mono- and dual-therapy regimens, lack of child-friendly formulations, defaulting ART and limited availability of approved ARVs for children (10).

The WHO previously recommend paediatric first-line ART regimens that include a boosted protease inhibitor (PI) combined with two nucleotide reverse transcriptase inhibitors (NRTIs) for children under 3 years of age (8). As of 2019, the paediatric first- line ART regimen has undergone change with the inclusion of INST's such as DTG (12) as depicted in Figure 1. NNRTIs have fallen out of favour as the backbone of paediatric ART due to the high levels of resistance to NNRTIs secondary to PMTCT (13)(4)(14). A meta-analysis found that PDR prevalence in children in sub-Saharan Africa is very high in PMTCT-exposed and PMTCT-unexposed children (14). In the PMTCT-unexposed group, prevalence of PDR increased markedly over time (14). The pooled PDR prevalence in African children is 4–15 times higher than that reported in adults (9).

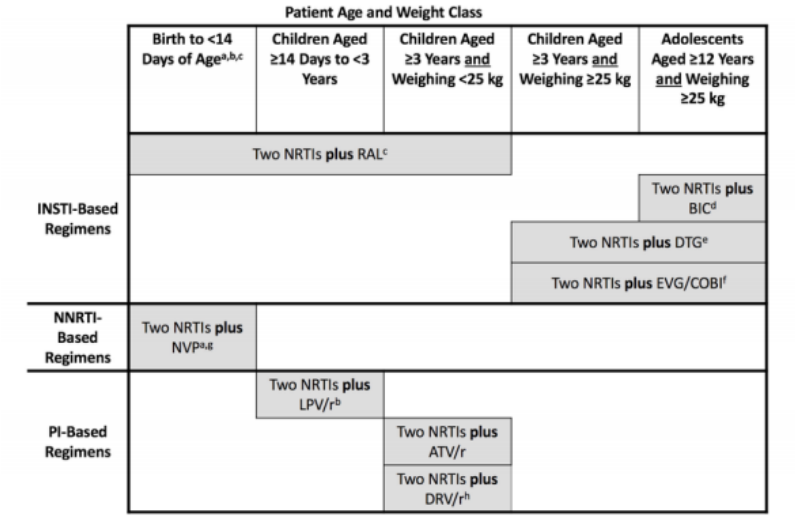

Figure 1: 2019 WHO ART paediatric recommendations according to patient weight and age

An analysis from five sub-Saharan African countries found that 785 (54%) of 1450 children (median age 18 months) living with HIV (CLWH) had PDR to 1 or more ARVs prior to treatment initiation (13). The commonest mutations found were to the NNRTI class, present in 53% of cases. Levels of resistance to EFV or NVP were 53% (range: 34% in Swaziland to 64% in Zimbabwe), and the prevalence of NRTI mutations was 9% (13). The commonest mutations to NNRTI were in positions 181 and 103 (13). The K103N and M184V mutations were more frequent in South Africa than in other countries, which could be explained by earlier and/or programmatic use of EFV and lamivudine or emtricitabine (3TC/FTC) and possibly by the earlier introduction of Option B+ as a PMTCT strategy (10). Resistance to PIs in infants and young children newly diagnosed with HIV in sub-Saharan countries is low (<3%), probably due to low rates of maternal PI use (6).

Based on the high prevalence of PDR in children (13), the WHO currently recommends that all paediatric patients should have a genotyping resistance test prior to initiation of ART and on changing to second-line (7). This is frequently done in high-middle income countries but in low-income/resource limited countries such as South Africa, routine resistance testing is rarely performed due to the cost and complexities of resistance

genotyping limiting it to children that are failing second-line regimens (9). In South Africa, the current guidelines from the National Department of Health recommend resistance testing in children when there is virological failure after more than 2 years of being on a PI or an integrase inhibitor based regimen (15).

HIV Drug resistance poses a major public health challenge and impacts individual patient care as it reduces the number of available and effective treatment options and threatens the success of both prevention and treatment programs such as the UNAIDS 90-90-90 global treatment targets (6). Understanding HIV drug resistance in the paediatric population is particularly important because children have higher viral load set points, with more rapid disease progression compared to adults (8).

Justification for this study

Understanding HIV resistance mutations in a population impacts on decisions around the choice of ART regimens and ensures sustainability and durability of these regimens. Despite South Africa having the world's largest ART programme (4), there is a paucity of data on the prevalence and types of resistance mutations in children and a need for more studies in this area. The aim of this study is to describe the resistance mutations of children attending a paediatric HIV clinic in a tertiary hospital. Furthermore, this study aims to determine the risk factors associated with certain mutations and the clinical outcomes of those with mutations compared to those without mutations.

AIMS AND OBJECTIVES

In this study, we aim to determine the frequency and timing of HIV resistance mutations in children and adolescents attending an ART clinic at Chris Hani Baragwanath Academic Hospital (CHBAH), Soweto, South Africa.

Objectives

1. To identify the five most prevalent resistance mutations affecting each of the NNRTI, NRTI and PI ARV drug groups.
2. To determine risk factors, including demographic and clinical characteristics, associated with the five most frequently encountered ARV mutations in each class of ARV drugs (case-control analysis).
3. To determine clinical outcomes of children and adolescents with documented HIV drug resistance, compared to children without drug resistance (case-control analysis and survival analysis).

METHODOLOGY

Study design

This will be a retrospective, case-control study of paediatric patients under the age of 15 years treated at Harriet Shezi Children's Clinic (HSCC) who were tested for HIV resistance from January 2011 to December 2020.

Study site

HSCC is located at CHBAH. The clinic was established in 2000 and serves the clinical and psychological needs of HIV-infected infants, children and adolescents. HSCC has one of the largest cohorts of HIV-infected children and adolescents, over 9000 to date (personal communication, Dr Sipambo). Since 2009, HSCC has transferred out stable, virally suppressed children and adolescents which has increased the clinic population of children and adolescents with more complex HIV disease, including those with opportunistic infections, side effects on ART, virologic failure and resistance to ART requiring second or third line ART.

From 2013 onwards, HSCC has focused on complicated HIV cases, serving as a referral site for surrounding clinics and hospitals. In 2014, HSCC established dedicated

Virologic Failure and Resistance clinics in order to offer differentiated care packages for this group of patients.

Additionally, HSCC manages complex paediatric and adolescent patients with co-morbidities that require specialist care from fields such as cardiology, renal, endocrine, pulmonology and oncology.

Study population

Paediatric patients seen at HSCC from 01 January 2011 to 31 December 2020, with available HIV drug resistance test results will comprise the focus group of this study (the DRT group). A comparator group of age-matched children that remained virologically suppressed on first-line ART and who did not require drug resistance testing at any stage during the course of their follow-up at HSCC will serve as controls against which to identify risk factors for the development of drug resistance (Control group).

Study subjects

1. Inclusion criteria

   Cases: Children and adolescents under the age of 15 years, who had an HIV drug resistance test, regardless of ART history or exposure during the study time period.

   Controls: Age-matched to cases (within 3 months to cases, for children aged <12 months; within 6 months to cases, for children ≥12 months of age), with suppressed viral load on first line ART and who therefore never underwent drug resistance testing.

2. Exclusion criteria

   Children and adolescents with no available HIV resistance test result, despite meeting the criteria for HIV drug resistance testing.

   Children and adolescents failing third line ART regimens.

STUDY METHODS

Demographic, clinical and laboratory data (Appendix) will be extracted from the HSCC REDCap database. REDCap is an online data bank onto which clinical data is routinely collected and recorded at HSCC on an ongoing basis. HIV drug resistance test results will be obtained, with permission, from the National Health Laboratory Service (NHLS) Corporate Data Warehouse.

Virologic failure will be defined as plasma viral load (VL) >1000 RNA copies/mL based on two consecutive viral load measurements 6 ± 3 months apart, with the first VL at least 6 months after ART initiation (16). First line and second line regimens will be defined according the National Department of Health paediatric regimen (Table 1).

Prior to 2018, HSCC performed drug resistance testing for all children failing first line therapy (NNRTI or PI based), second line, or third line ART regimens. From September 2018 onwards, DRTs were no longer performed on those failing first line NNRTI-based regimens. The reasons for the change in practice included cost effectiveness, predictable DRT results for those on NNRTI-containing ART regimens, and minimal impact on the choice of second line ART, specifically PI-based, and subsequent viral suppression. These changes also allowed for HSCC to be in-line with the National Department of Health recommendations on drug resistance testing.

Table 1: Summary of Paediatric First and Second Line ART Regimens - South African Department of Health Guidelines 2004 through 2019

<table>
<tr><th>Line of Therapy</th><th>Guideline</th><th>Children <3 years and <10 kg</th><th>Children ≥3 years and ≥10 kg</th><th>Adolescents 10-15 years and <40 kg</th><th>Adolescents ≥15 years and ≥40 kg</th></tr>
<tr><td rowspan="5">First Line</td><td>South Africa 2004</td><td>d4T + 3TC + LPV/r</td><td>d4T + 3TC + EFV</td><td>d4T + 3TC + EFV/NVP</td><td>d4T + 3TC + EFV/NVP</td></tr>
<tr><td>South Africa 2010</td><td rowspan="3">ABC + 3TC + LPV/r</td><td colspan="2" rowspan="2">ABC + 3TC + EFV</td><td rowspan="2">TDF + 3TC/FTC + EFV</td></tr>
<tr><td>South Africa 2015</td></tr>
<tr><td>South Africa 2019</td><td>If <20 kg: ABC + 3TC + LPV/r
If ≥20 kg: ABC + 3TC + DTG</td><td>If <20 kg: ABC + 3TC + LPV/r
If 20-35 kg: ABC + 3TC + DTG
If ≥35 kg: TDF + 3TC + DTG</td><td>Males: TDF + 3TC + DTG
Females of childbearing potential: TDF + 3TC/FTC + EFV</td></tr>
<tr><td rowspan="4">Second Line</td><td>South Africa 2004</td><td>AZT + ddI + NVP</td><td colspan="3">AZT + ddI + LPV/r</td></tr>
<tr><td>South Africa 2010</td><td rowspan="2">Refer to specialist</td><td>AZT + ddI + LPV/r</td><td>TDF + 3TC/FTC + LPV/r</td><td>AZT + 3TC + LPV/r</td></tr>
<tr><td>South Africa 2015</td><td colspan="3">AZT + 3TC + LPV/r</td></tr>
<tr><td>South Africa 2019</td><td>ABC or AZT + 3TC + LPV/r</td><td>If first line regimen contained NNRTI: 2 NRTIs + DTG
or
2 NRTIs + LPV/r</td><td>If first line regimen contained NNRTI: 2 NRTIs + DTG
or
2 NRTIs + LPV/r</td><td>If first line regimen contained NNRTI: AZT + 3TC/FTC + DTG
or
AZT + TDF + 3TC/FTC + DTG
or
TDF + 3TC + LPR/r</td></tr>
</table>

Data management and analysis

The Stanford HIV drug resistance database (17) is routinely used to identify HIV drug resistance mutations and generate resistance profiles for each participant that undergoes HIV resistance testing at HSCC. For each ARV drug evaluated in the assay, a report is automatically generated as to whether the tested HIV strain is susceptible, intermediate or resistant. Extracted data will be entered into a data collection sheet (Appendix). Data will be entered into an Excel spreadsheet on a password protected computer accessible only by the principal investigator. Statistica® and R version 4.0.4 (18), will be used to analyse the recorded data. Continuous data will be summarized using the mean and standard deviation (SD) if normally distributed and median or interquartile range (IQR) if the distribution is skewed. Categorical data will be expressed as frequencies and proportions. A confidence interval of 95% will be used in all data analysis.

In the case-control analysis, univariate and multivariate logistic regression models will be developed to determine risk factors for the development of HIV drug resistance in those failing ART, compared to children that remained virologically suppressed on their first line regimen. Risk factors that will be considered include: sex, age at HIV diagnosis, age at ART initiation, first line ART regimen used (NNRTI or PI containing), exposure to PMTCT, type of PMTCT exposure, baseline CD4 count, baseline HIV VL result, previous or current anti-tuberculosis treatment, and orphan status of the child.

A survival analysis will be conducted to compare the outcomes between children with and without virologic failure, to compare survival outcomes between children with different ART class drug mutations. Kaplan-Meier curves will be used to compare outcomes and Cox proportional hazards analysis will be undertaken to compare hazard ratios between groups.

Assistance with statistical analysis will be provided by the Faculty of Health Sciences Biostatistics Department and by Prof Moore.

Sample size calculation

Sample size estimates were calculated using the epiR package (19)(18). Using a case control study design, we will quantify the association between exposure to rifampicin-containing TB treatment and virological failure as a major risk factor for the development of ART resistance. Assumptions for the sample size calculation are: 1) 43% of children without drug resistance testing had tuberculosis at any stage during their course of ART (20); 2) for a matched case control study, the correlation between case and control exposures for matched pairs (Rho) is minimal (0.2) to moderate (0.60); 3) a 2-fold increased odds of virological failure in children and adolescents treated with rifampicin-containing TB therapy, compared to those that never received anti-TB treatment.

Assuming an equal number of cases and controls, 328 to 612 children need to be enrolled into the study to detect an odds ratio of 2.0 with 0.80 power using a two-sided alpha of 0.05 using a matched case-control study design (Table 2).

Table 2: Sample Size Calculation for Matched Case-Control Analysis

Total	Case	Controls	Power	Two-sided P-value	Rho	Odds Ratio
328	164	164	0.8	0.05	0.20	2
348	174	174	0.8	0.05	0.25	2
372	186	186	0.8	0.05	0.30	2
398	199	199	0.8	0.05	0.35	2
428	214	214	0.8	0.05	0.40	2
462	231	231	0.8	0.05	0.45	2
504	252	252	0.8	0.05	0.50	2
552	276	276	0.8	0.05	0.55	2
612	306	306	0.8	0.05	0.60	2

Using an unmatched case control study design, 266 children (133 cases and 133 controls) need to be enrolled into the study to detect an odds ratio of 2.0 with 0.80 power using a two-sided alpha of 0.05.

Ethical considerations

As this is a retrospective study on routinely collected clinical and laboratory data, a waiver of informed consent is requested. Confidentiality will be maintained through use of a password protected computer, accessible only by the principle investigator. Furthermore, although identifier details (names, surnames, hospital numbers and dates of birth) will be required to link between the HSCC database and the NHLS database (for HIV drug resistance test results) all identifying parameters will be removed from the database that will be used for analysis.

The study protocol will be submitted to the Human Research Ethics Committee (Medical) at the University of Witwatersrand for approval. Permission to conduct the study will be obtained from the CHBAH Medical Advisory Committee, the head of HSCC, the head of the Department of Paediatrics and Child Health at CHBAH, and the NHLS Ethics Committee (for permission to access HIV drug resistance test results). Dr Kim Steegen has been identified as a co-author from the NHLS in the study.

Timeline

	01/20– 11/20	02/20-02/21	03/21 – 08/21	09/21– 10/21
Literature review				
Preparing Protocol				
Protocol assessment				
Protocol Revisions				
Ethics Application				
Collecting data				
Data Analysis				
Writing up – thesis				
Writing up Paper				

Funding

The researcher will fund this study: stationary and printing costs are estimated to amount to R 2500. The University of the Witwatersrand Faculty of Health Sciences will be approached to assist with publication costs of any journal articles that emanate from this research.

Limitations

The study is a retrospective study and depends on routinely collected data which may be incomplete. HIV drug resistance test results from 2014 onwards have not routinely been captured into the HSCC REDCap database: the assistance of Dr Kim Steegen from the NHLS will be sought to access all HIV DRM results from 2014 through 2020.

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Data collection sheet

Study number	
Sex	
Age	
Age of ART start	
PMTCT exposure (Y/N)	
PMTCT regimens	
CD4 counts	
1. Baseline CD4	
1. Baseline CD4 Age	
2. Second CD4	
2. Interval from last CD4	
3. Third CD4	
3. Interval from last CD4	
4. Fourth CD4	
4. Interval from last CD4	
5. Fifth CD4	
5. Interval from last CD4	
6. Sixth CD4	
6. Interval from last CD4	
7. Seventh CD4	
7. Interval from last CD4	
8. Eighth CD4	
8. Interval from last CD4	
9. Ninth CD4	
9. Interval from last CD4	
10. Tenth CD4	
10. Interval from last CD4	
11. Last CD4	
11. Interval from last CD4	
Risk Factors	
Previous TB Rx (Y/N)	
TB Rx at time of DRT (Y/N)	
Date of TB Rx start	yyyy/mm/dd
Orphaned (Y/N)	
If orphaned - type (M/F/B)	

First ART Regimen	
Drugs in 1st regimen	
Second ART regimen	
Interval on treatment commenced.	
Drugs in 2nd regimen	
VL measurements	
1. Baseline VL	
1. Baseline VL Age	
2. Second VL	
2. Interval from last VL	
3. Third VL	
3. Interval from last VL	
4. Fourth VL	
4. Interval from last VL	
5. Fifth VL	
5. Interval from last VL	
6. Sixth VL	
6. Interval from last VL	
7. Seventh VL	
7.Interval from last VL	
8. Eighth VL	
8. Interval from last VL	
9. Ninth VL	
9. Interval from last VL	
10. Tenth VL	
10. Interval from last VL	
11. Last VL	
11. Interval from last VL	
Date of DRT	
First DRT	yyyy/mm/dd
PI Mutation list	
Reverse transcriptase	
Mutation list	
Second DRT	yyyy/mm/dd
PI mutation list	
Reserve transcriptase	
Mutation list	

Data collection sheet

		Outcome	
		Survived/died/LTFU/TFOut	
		Outcome Date	

M=mother; F=Father; B=Both

APPENDIX 3: Ethics Approval Certificates

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

R14/49 Dr Zinhle Vilakazi

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)

CLEARANCE CERTIFICATE NO. M210526

NAME: (**Principal Investigator**)	Dr Zinhle Vilakazi
DEPARTMENT:	Paediatrics Harriet Shezi HIV Children's Clinic Chris Hani Baragwanath Academic Hospital
PROJECT TITLE:	Patterns of HIV resistance in children attending an antiretroviral clinic in Soweto, South Africa
DATE CONSIDERED:	28/05/2021
DECISION:	Approved unconditionally
CONDITIONS:	
SUPERVISOR:	Dr Nosisa Sipambo and Prof David Moore
APPROVED BY:	CB Penny Dr CB Penny, Chairperson, HREC (Medical)
DATE OF APPROVAL:	05/07/2021

This clearance certificate is valid for 5 years from date of approval. Extension may be applied for.

DECLARATION OF INVESTIGATORS

To be completed in duplicate and **ONE COPY** returned to the Research Office Secretary on the Third Floor, Faculty of Health Sciences, Phillip Tobias Building, 29 Princess of Wales Terrace, Parktown, 2193, University of the Witwatersrand. I/we fully understand the conditions under which I am/we are authorized to carry out the above-mentioned research and I/we undertake to ensure compliance with these conditions.Should any departure be contemplated, from the research protocol as approved, I/we undertake to resubmit the application to the Committee. **I agree to submit a yearly progress report.** The date for annual re-certification will be one year after the date of convened meeting where the study was initially reviewed. In this case, the study was initially reviewed in **May** and will therefore be due in the month of **May** each year. Unreported changes to the application may invalidate the clearance given by the HREC (Medical).

Principal Investigator Signature

Date

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES

Academic Affairs and Research
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12 August 2021

Applicant: Zinhle Vilakazi
Institution: University of the Witwatersrand
Department: Paediatrics
Email: mandisa2109@gmail.com
Cell: 078 369 3941

Re: Approval to access National Health Laboratory Service (NHLS) Data

Your application to undertake a research project **"HIV resistance patterns in children attending an antiretroviral clninc in Soweto, South Africa, Ref No: PR2119005"** using data from the NHLS database has been reviewed. This letter serves to advise that the application has been approved and the required data will be made available to you ***without patient names*** to conduct the proposed study as outlined in the submitted application. Submissions should be made annually on the AARMS system – https://aarms.nhls.ac.za.

Please note that approval is granted on your compliance with the NHLS conditions of service and that the study can only be undertaken provided that the following conditions have been met.

- Processes are discussed with the relevant NHLS departments (i.e. Information Management Unit and Operations Office) and are agreed upon.
- Confidentiality is maintained at participant and institutional level and there is no disclosure of personal information or confidential information as described by the NHLS policy.
- NHLS Data cannot be used to track patients as no pre-approval/consent is obtained from Patients.
- All data requested should be in accordance with the research protocol submitted and approved by the relevant Ethics Committee.
- Request for the inclusion of the NHLS as a source of data in the original protocol to be approved by Ethics as NHLS does not have a Human Research Ethics Committee.
- A final report of the research study and any published paper resulting from this study are submitted and addressed to the NHLS Academic Affairs and Research office and the NHLS has been acknowledged appropriately.

Please note that this letter constitutes approval by the NHLS Academic Affairs and Research Office. Any data related queries may be directed to NHLS Corporate Data Warehouse, contact number: 011 386 6074 email: zarina.sabat@nhls.ac.za

Dr Babatyi Malope-Kgokong
National Manager: Academic Affairs and Research