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HIV drug resistance in children and adolescents on NNRTI-based antiretroviral therapy and subsequent virologic response to dolutegravir-based regimens in Ghana

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Abstract

HIV drug resistance (HIVDR) was retrospectively characterized among 20 children and adolescents with HIV with virologic failure on non-nucleoside reverse transcriptase inhibitor (NNRTI)-based therapy, and virologic response in those switched to dolutegravir (DTG)-based therapy described. All participants had at least one NNRTI resistance mutation, most commonly K103N ($N=12$) and 15 (75%) had nucleoside reverse transcriptase inhibitor (NRTI) resistance mutations, most commonly M184I/V ($N=15$). Five (45%) of 11 participants who were switched to DTG-based regimens for a median of 50 months had HIV suppression. Further studies to understand the role of pre-existing HIVDR in the failure of DTG-based regimens are needed.

Keywords NNRTIs, HIV drug resistance, Children and adolescents, Dolutegravir, Antiretroviral therapy, Ghana

Background

Human immunodeficiency virus (HIV) drug resistance (HIVDR) poses a challenge to achieving UNAIDS global targets [1]. For decades, children have been treated with inappropriate adult antiretroviral formulations or poorly tolerated pediatric formulations with high toxicity and a low genetic resistance barrier without adequate virologic monitoring [2, 3]. Therefore, children and adolescents with HIV (CAWH) experience a high frequency of drug resistance mutations (DRMs) after failing first-line regimens [4–6]. As HIV treatment programs in resource-limited settings transition from non-nucleoside reverse transcriptase inhibitor (NNRTI)-based to dolutegravir (DTG)-based regimens, often initiated in the absence of drug resistance testing to optimize nucleoside reverse transcriptase inhibitors (NRTIs) backbones, there is concern that a significant proportion of antiretroviral

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therapy (ART)-experienced CAWH could be on functional monotherapy [7]. The current study examined the frequency and pattern of HIVDR mutations in Ghanaian CAWH with virologic failure on NNRTI-based regimen at Korle Bu Teaching Hospital prior to the introduction of DTG in Ghana and described the subsequent virologic response in those switched to DTG-based ART.

Methods

Participants included CAWH from a previous study that examined the virologic response to NNRTI-based ART from the Pediatric HIV/AIDS Care Program at Korle-Bu Teaching Hospital in Ghana [8]. Genotypic drug resistance testing of stored samples from the 96 individuals with HIV-1 RNA > 1,000 copies/ml was attempted [8]. Briefly, frozen plasma samples were shipped on dry ice to the Virology Quality Assurance-approved laboratory [9] at the Providence-Boston Center for AIDS Research Laboratory. RNA extraction was performed using the Qiagen EZ1 Advanced XL and DSP Virus Kit. HIV-1 *pol* was amplified using two-round PCR, and next-generation sequencing (NGS) was conducted on the Illumina MiSeq platform following previously published protocols [10–12]. Consensus sequences were assessed using the hivmmer bioinformatics pipeline to capture potentially emerging resistance while maintaining analytical rigor. Drug resistance analysis utilized the Stanford University Drug Resistance Database (version 9.5.1) for interpretation [13]. Predicted resistance was considered clinically relevant at Stanford intermediate-level resistance or higher. DRMs were categorized as major mutations (≥ 20% frequency) or minority resistance variants (2–<20% frequency). HIV-1 subtyping was performed using REGA v3. Analyses included an assessment of resistance to medications taken at the time of genotyping and potential alternative treatment options including tenofovir, rilpivirine, etravirine, and doravirine.

Descriptive statistics of demographic, clinical, and drug resistance data were performed using Stata MP version 18.5. Continuous variables were summarized by median with interquartile ranges, while categorical variables were reported as counts and percentages. Ethical approval was obtained from the Institutional Review Board of Korle Bu Teaching Hospital (KBTH-IRB/00060/2017). Written informed consent, including permission to store samples for HIVDR testing was obtained from the parents or legal guardians at enrollment.

For participants who were switched to DTG-based regimens, routine viral load was performed at follow-up per standard clinical protocols. The most recent viral load was used to assess virologic suppression. HIVDR or INSTI resistance testing was not performed at the time of DTG initiation or treatment failure.

Results

Study population

Twenty samples were successfully amplified and sequenced (Figure S1). Of the 20 participants, the median (range) age was 10 (2–15) years and 60% were female. The ART regimen at the time of failure is shown in Table S1. Fifteen participants (75%) had CRF02_AG (Table S1).

HIV-1 drug resistance mutations following virologic failure on NNRTI-based ART

All participants (100%) had at least one DRM; 100% NNRTIs, 75% NRTIs (Fig. 1A & B). M184I/V ($N = 15$) and NNRTI-associated K103N ($N = 12$) were the most common DRMs. Thymidine analogue mutations (TAMs) were observed in three participants: M41L in one participant and T215SY in two participants. The K65R mutation, which reduces susceptibility to tenofovir was not detected in any participant. Among NNRTI mutations, K103N was predominant, followed by Y181C and G190A. NNRTI minority resistance variants including Y181S, K103N, V179D, K101E, Y188F, G190S, M230I and L234I were observed in nine participants, with NGS frequencies ranging from 2 to 9% (Fig. 1D).

Resistance prediction to current and alternative treatment options

All participants exhibited high-level resistance to nevirapine and 17 (85%) of 20 had high-level resistance to efavirenz (Figure S2). Overall, 14 had high-level lamivudine resistance, whilst 6 and 11 participants retained susceptibility to abacavir and zidovudine, respectively. Five participants had resistance to all NRTIs and NNRTIs in the regimens. Regarding alternative treatment options for unexposed NRTIs, 15 participants were predicted as susceptible to tenofovir. Predicted intermediate- to high-level resistance to etravirine, rilpivirine and doravirine was found in 6 (30%), 8 (40%) and 12 (60%) of 20 participants, respectively (Figure S2).

Treatment outcome on dolutegravir-based ART

Eleven participants who remained in care at our center were switched to DTG-based ART following its introduction and a change in HIV treatment guidelines in Ghana (Table 1). After 30–59 months of DTG-based ART, 5 (45%) participants had HIV suppression (HIV RNA < 1000 copies/mL). Based on DRMs, two participants (6 and 9) retained susceptibility to all three drugs in their DTG-based regimen, while six were on functional dual therapy and two were effectively on DTG functional monotherapy (Table 1).

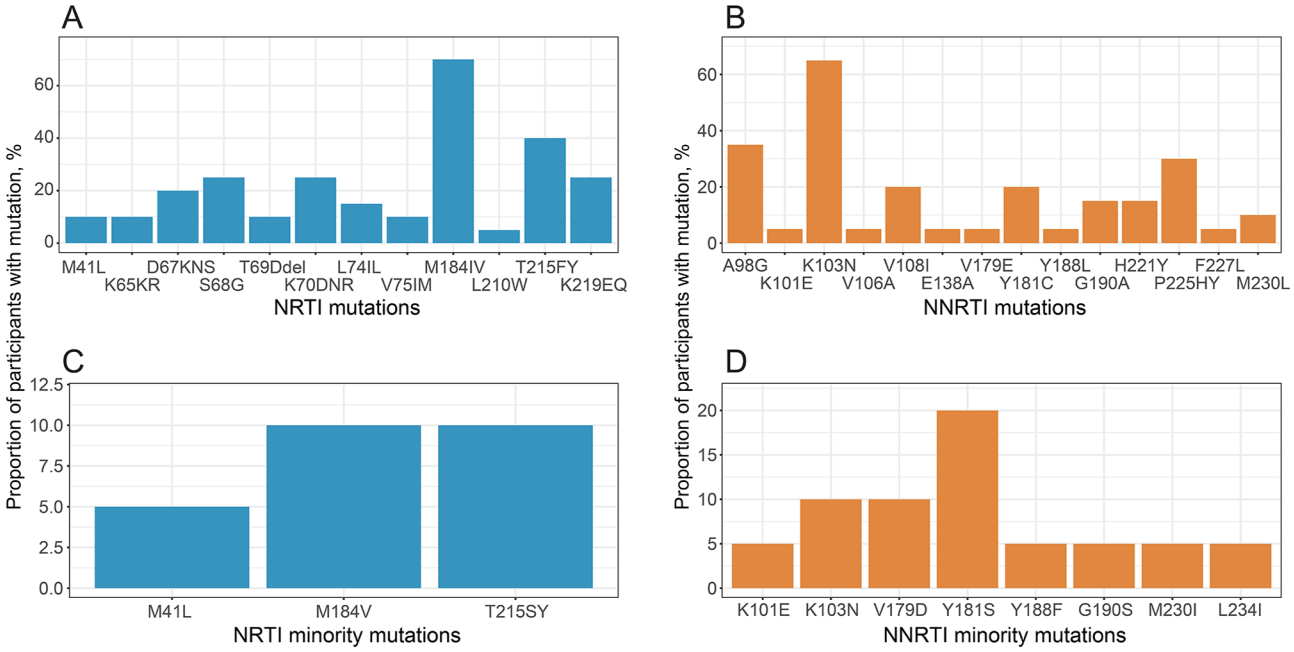

Fig. 1 Drug resistance mutations (DRMs) in 20 children and adolescents on non-nucleoside reverse transcriptase inhibitor (NNRTI)-based antiretroviral therapy in Ghana. Panel A shows the major nucleoside reverse transcriptase inhibitor (NRTI) DRMs, Panel B shows the major NNRTI DRMs, Panel C shows the NRTI minority variants and Panel D shows the NNRTI minority variants

Discussion

We observed a frequency of DRMs among 20 Ghanaian CAWH who had virologic failure on NNRTI-based ART [8]. Although genotyping was successful in only 21% of the individuals with virologic failure, this high HIVDR prevalence is similar to findings from other African countries, where 80–93% of CAWH failing first-line NNRTI-based regimens show resistance mutations [4, 5, 10–12]. With limited routine virologic monitoring and second-line ART options, CAWH have likely been exposed to failing regimens for decades. Paediatric HIV treatment programs in Africa have now moved away from NNRTIs to DTG-based ART, but the availability of effective NRTI options remains unchanged.

Three-quarters of participants had intermediate- to high-level resistance to lamivudine, a key component of ART regimens. The NRTI-associated M184I/V mutations were most common, resulting in resistance to lamivudine or emtricitabine, which are components of the Ghana national and WHO-recommended DTG-based regimens [13, 14]. Notably, thymidine analogue mutations (TAMs) were detected in three participants, including M41L in one participant and T215SY in two participants. These TAMs are concerning as they can confer cross-resistance to multiple NRTIs. In the setting of second-line DTG-based ART, a zidovudine plus lamivudine backbone with pre-existing TAMs may not adequately protect against the emergence of DTG resistance [15, 16].

A particularly concerning finding was the low virologic suppression rate of 45% among participants on DTG-based regimen after 30–59 months. This rate is similar to the 43% reported among adolescents who achieved HIV RNA < 400 copies/mL at 144 weeks in IMPAACT P1093 [17] but lower than the 89% suppression among treatment-experienced CAWH in ODDESSEY [15]. The switch to DTG-based ART in the setting of viremia and HIVDR may have contributed to the poor virologic response. First, the high prevalence of pre-existing NRTI resistance at the time of the switch may have compromised the effectiveness of DTG-based regimens, as some participants were effectively on functional DTG monotherapy or dual therapy. Second, though not directly assessed here, adherence challenges common in paediatric populations may have played a role [18]. Third, the lack of resistance testing before switching to DTG-based regimens prevented optimizing background regimens, potentially compromising treatment efficacy [19].

Regarding alternative treatment options, three-quarters of participants remained susceptible to tenofovir, suggesting its potential value in future regimens for older children and adolescents. Some participants remained susceptible to zidovudine and abacavir, key components of pediatric ART regimens for younger children and neonates even currently in settings like Ghana; knowledge of this is only available through drug resistance testing. The high detected prevalence of K103N and Y181C is worrisome, as these mutations can confer cross-resistance to newer generation NNRTIs, including rilpivirine, which together with cabotegravir is part of the long-acting injectable treatment and holds high potential to improve

Table 1 Clinical and drug resistance data of 20 the participants

	Drug Regimen at VF	Age at NNRTI VF (years)	NRTI resistance before switch to DTG	NNRTI resistance before switch to DTG	Duration on DTG (months)	Viral Load after switch to DTG
1	ABC/3TC/NVP	13	M184V, T215Y	A98G, K103N, P225H	50	207,000
2	ZDV/3TC/EFV	14	None	K103N, P225H	70	24,700
3	ZDV/3TC/EFV	11	M41L, V75M, M184V, L210W, T215Y	A98G, K103N, P225H, F227FL	66	11,100
4	ZDV/3TC/EFV	17	V75I, M184V	K103N, P225H	54	3,420
5	ZDV/3TC/EFV	12	S68G, M184V, T215F	A98G, K103N, V108I	53	2,460
6	ABC/3TC/EFV	15	None	Y188L	60	1,030
7	ZDV/3TC/NVP	17	S68G, M184V, T215F, K219Q	K103N, E138A, M230L	65	145
8	ZDV/3TC/EFV	14	S68G, M184V	K103N, V179E, H221Y	12	64
9	ZDV/3TC/EFV	11	None	Y181C	66	55
10	ZDV/3TC/NVP	10	S68G, M184V	V106A	64	40
11	ZDF/3TC/NVP	13	D67N, K70R, M184V, T215F, K219E	Y181C, G190A	49	40
12	ZDV + 3TC + EFV	15	K70R, M184V	A98G, K103N, P225H	Not switched	Not switched
13	ZDV + 3TC + EFV	16	None	G190GA	Not switched	Not switched
14	ZDV + 3TC + EFV	9	S68G	K103N	Not switched	Not switched
15	ABC + 3TC + NVP	13	K65KR, D67KN, T69D, K70R, L74I, M184V, T215F, K219Q	A98G, V108I, Y181C, H221Y	Not switched	Not switched
16	ZDV + 3TC + EFV	12	K65KR, D67KN, T69del, K70DN, L74LI, M184V, T215F, K219EQ	A98AG, K101KE, V108I, Y181C, G190GA, H221HY	Not switched	Not switched
17	ZDV + 3TC + NVP	10	M184I	K103N, M230L	Not switched	Not switched
18	ZDV + 3TC + NVP	2	NONE	K103N	Not switched	Not switched
19	ZDV + 3TC + NVP	15	M41L, D67S, K70R, L74I, M184V, T215F, K219Q	A98G, K103N, P225H	Not switched	Not switched
20	ZDV + 3TC + EFV	10	M184V	Y181C, G190A	Not switched	Not switched

Footnote: Abbreviations: *ABC*, Abacavir, *EFV*, Efavirenz, *3TC*, Lamivudine; *NVP*, Nevirapine; *ZDV*, Zidovudine; *DTG*, Dolutegravir; *TDF*, Tenofovir. The minimum period on DTG-based ART was 12 months. The HIV-1 pol gene, which was amplified and sequenced in this study, encompasses the protease (PR) and reverse transcriptase (RT) regions. INSTI resistance was not evaluated as integrase sequencing was not performed

adherence in youth. With 40% of participants exhibiting pre-existing cross-resistance to rilpivirine, the role of injectable cabotegravir/rilpivirine will be limited. Etravirine showed potential activity, with lower resistance rates (30%) compared to other second-generation NNRTIs (rilpivirine-40%; doravirine-60%). While this suggests potential utility in ART-experienced children, limited availability in resource-constrained settings, lack of child-friendly formulations, and adherence concerns due to twice-daily dosing may limit its use. Further research, through larger prospective studies, is needed to confirm its effectiveness in children with prior NNRTI exposure, particularly in contexts where DTG-based regimens have shown suboptimal outcomes.

This study has several limitations that should be considered when interpreting its findings. First, adherence was not directly assessed, which is a critical factor in virologic failure; resistance mutations may have resulted from poor ART adherence rather than pre-existing resistance alone. Second, only 20 of 96 samples with viral load > 1000 copies/mL were successfully sequenced, likely due to RNA degradation (data not shown), limiting the ability to conduct association studies and the generalizability of findings. Third, resistance testing was not available to optimize the NRTI backbone at switch to DTG and resistance testing was not available at the time of virologic failure on DTG-based ART. Thus, resistance profiles upon failure of DTG-based regimens are unknown. Despite these limitations, the study provides valuable insights into pediatric HIV treatment challenges in resource-limited settings, reinforcing the importance of drug resistance testing, adherence monitoring, and optimized regimen selection to improve treatment outcomes.

Abbreviations

ART	Antiretroviral Therapy
CAWH	Children and Adolescents With HIV
DRM	Drug Resistance Mutation
DTG	Dolutegravir
HIV	Human Immunodeficiency Virus
HIVDR	HIV Drug Resistance
NGS	Next Generation Sequencing
NNRTI	Non-nucleoside Reverse Transcriptase Inhibitor

NRTI	Nucleoside Reverse Transcriptase Inhibitor
PCR	Polymerase Chain Reaction
RNA	Ribonucleic Acid
WHO	World Health Organization
UNAIDS	Joint United Nations Programme on HIV/AIDS

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12981-025-00762-4.

Supplementary Material 1

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Author contributions

Conceptualization, AA and AK; Methodology, AA, AK RK, VN, YA; formal analysis, AA, AK, RK, VN, YA investigation AA, AK; resources, AA, AK; data curation;, AA, AK, RK, VN, JH; writing-original draft preparation, AA, AK, RK, VN; writing, AA, AK, RK, VN, KS; reviewing and editing, AA, AK, RK, VN, YA, JD, JH, KS, CM, ML, BG; visualization, AA, AK, RK, VN supervision, AK, RK; project administration, AA, AK; funding acquisition, AK. All authors have read and agreed to the published version of the manuscript.

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Data availability

No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate

The study was conducted in accordance with the Declaration of Helsinki and approved by the Institutional Review Board of Korle Bu Teaching Hospital ((KBTH-IRB/ 00060/2017) for studies involving humans. Written informed consent, including consent to store and use samples for HIVDR testing, was obtained from the parents or legal guardians of each minor participant before enrolment in the study.

Consent for publication

Not applicable.

Conflicts of interest

The authors declare no conflicts of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

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