Genotypic resistance profiles in HIV-infected children experiencing first-line treatment failure in Southern Ethiopia

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EXECUTIVE SUMMARY

- Clinical monitoring of HIV infection in resource-limited settings is a major challenge due to lack of availability of routine viral load monitoring and genotypic drug resistance testing
- Regimens are frequently changed without knowledge of resistance
- In Ethiopia, drug resistance testing is not routinely available, more than a decade after the widespread roll-out of cART
- The burden and characteristics of drug resistance among adults failing first-line cART has been described, however resistance burden in HIV-infected Ethiopian children failing first-line cART remains understudied
- In 90 children experiencing virologic failure of first-line cART, 73 (81%) harboured drug resistance
- 42% of resistant participants harboured resistance to all four NRTIs available for second-line use
- Longer cART duration and prior regimen changes were significantly associated with detection of drug resistance mutations
- Replicate genotyping increased the breadth of resistance detected in 34% of participants

METHODS

STUDY POPULATION

- N= 780 children (<18 years of age) on or initiating first-line cART were recruited to the Ethiopia Pediatric HIV Cohort (EPHIC) in Southern Ethiopia[1]
- Clinical and sociodemographic characteristics were collected at baseline (enrolment)
- Children were assessed semi-annually for first-line treatment failure using WHO clinical, immunologic and virologic criteria
- N= 94 (12%) EPHIC participants experiencing virologic failure of first-line cART (pVL > 2,500 copies/mL) as of February 2017 were included in the present study

DRUG RESISTANCE GENOTYPING

- Total nucleic acids were extracted from dried blood spots (up to 2 extractions/participant; 2 spots/extraction)
- HIV-1 Protease and at least Reverse Transcriptase codons 1-234 were amplified using PCR with and without an initial Reverse Transcriptase step using up to 4 primer sets (1 primary, 3 alternate)
- The Stanford Drug Resistance Database HIVdb Program was used to investigate the presence of drug resistance mutations conferring resistance to Protease Inhibitors (PIs), Nucleoside Reverse Transcriptase Inhibitors (NRTIs), and Non-nucleoside Reverse Transcriptase Inhibitors (NNRTIs)
- Resistance to individual drugs was also determined using the Stanford Drug Resistance database where any level of reduced susceptibility was considered 'resistant'
- Drug resistance in each sequence was evaluated individually
- For each participant for whom >1 sequence was available, an inclusive consensus (*i.e.* a sequence that incorporates all observed polymorphisms at all sites) was generated and interpreted to capture the full breadth of resistance in the individual
- Hypermutated or otherwise defective sequences were excluded from drug resistance interpretation

FUNDING AND REFERENCES

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[1] Tadesse et al, 2017, PMID: 28246135

RESULTS

CHARACTERISTICS OF CHILDREN FAILING FIRST-LINE CART

Variable			Summary Statistic	N
Age at baseline, years Median [IQR]			12 [9-14]	94
Gender, % male			60%	94
pVL at baseline, log_{10} copies/mL Median [IQR]			3.9 [3.6- 4.6]	94
pVL at failure, log_{10} copies/mL Median [IQR]			4.2 [3.8- 4.7]	94
CD4+ T-cell count at baseline, cells/mm^3 Median [IQR]			500 [247- 781]	92
CD4+ T-cell count at ART initiation, cells/mm^3 Median [IQR]			276 [163- 613]	89
ART duration at baseline, months Median [IQR]			35 [18- 70.5]	89
Weight for age at baseline, Z-score* Median [IQR]			-1.5 [-2.1- (-0.6)]	89
Height for age at baseline, Z-score* Median [IQR]			-1.3 [2.1- (-0.4)]	89
ART regimen at baseline, % patients				
NRTI				93
	3TC+	AZT	66%	
		d4T	29%	
		TDF	3%	
		ABC	2%	
NNRTI				93
		EFV	22%	
		NVP	77%	
WHO Clinical Stage at baseline, % patients				
		Stage 1	85%	94

Table 1: Clinical and sociodemographic characteristics of 94 EPHIC participants experiencing virologic failure of first-line cART. Weight and Height for Age were measured using WHO Anthropomorphic Software.

Sociodemographic and clinical characteristics of the participants experiencing virologic failure of first-line cART resembled those of the larger EPHIC cohort[1]

HIGH PREVALENCE OF HIV-1 DRUG RESISTANCE

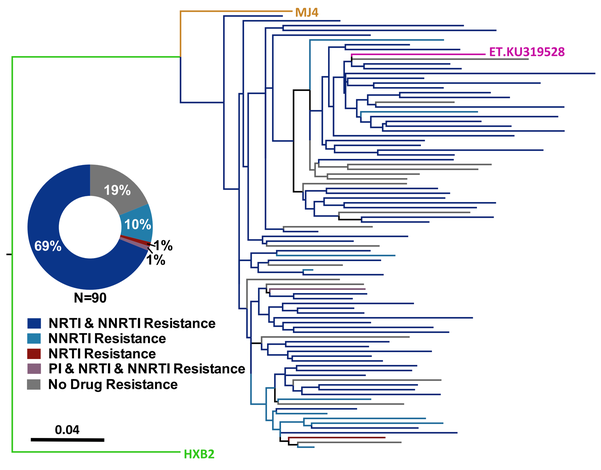

Figure 2: Prevalence of HIV-1 drug resistance among Ethiopian children experiencing virologic failure of first-line cART

At least 1 intact, non-hypermutated sequence was isolated from 90/94 participants (96%). A maximum-likelihood phylogeny was inferred from the inclusive consensus sequence for each participant. All sequences were subtype C, nesting separately from the Botswanan reference sequence MJ4 in a distinct sub-clade with the published Ethiopian sequence ET.KU319528.

Overall, 81% (73/90) participants harboured at least 1 drug resistance mutation: 69% (N=62) to both NRTIs and NNRTIs, 10% (N=9) to NNRTIs alone, 1% (N=1) to NRTIs alone and 1% to PIs, NRTIs, and NNRTIs.

No drug resistance was observed in the remaining N=17 (19%) participants.

DISTRIBUTION OF NRTI AND NNRTI RESISTANCE MUTATIONS

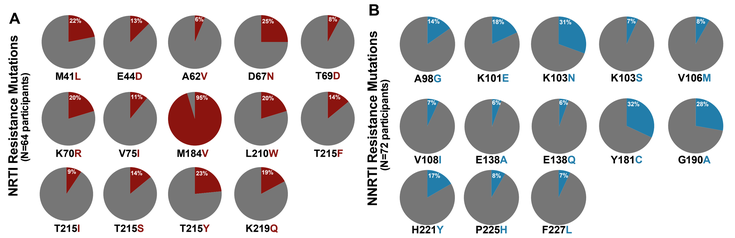

Figure 2: Prevalence of NRTI (panel A) and NNRTI (panel B) resistance mutations in participants with resistant genotypes. All mutations observed in >5% of resistant participants are shown.

Consistent with rapid selection by widely-used 3TC, M184V was the most common NRTI mutation, found in 61/64 (95%) NRTI-resistant participants. AZT- and D4T-associated D67N, T215Y, M41L, K70R and L210W were also commonly observed (20-25%).

Y181C and K103N were the most commonly observed NNRTI resistance mutations, observed in 32% and 31% of NNRTI-resistant participants, respectively

RESISTANCE SUBSTANTIALLY COMPROMISES WHO-RECOMMENDED FIRST- AND SECOND-LINE REGIMENS

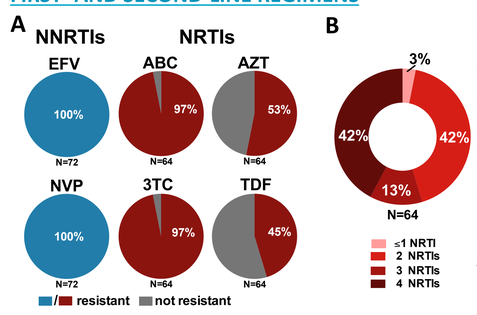

Figure 3: Implications of resistant genotypes on recommended first- and second-line regimens. **(A)** prevalence of resistance to recommended first-line NNRTIs and NRTIs, **(B)** burden of multi-NRTI resistance among participants harboring NRTI resistance (N=64).

Among NRTI-resistant participants: 97% harboured at least some resistance to each of 3TC and ABC, while 53% and 45% were resistant to AZT and TDF respectively (**A, red**)

100% of NNRTI-resistant participants were resistant to the two 2 WHO-recommended NNRTIs (**A, blue**).

42% (N=27) of NRTI-resistant participants harboured resistance to 2 of 4 WHO-recommended first- and second-line NRTIs, while 13% (N=9) were resistant to 3 of 4 recommended drugs. Strikingly, 42% harboured resistance to all four recommended NRTIs, substantially compromising second-line therapies for many participants (**panel B**).

FACTORS ASSOCIATED WITH VIROLOGIC FAILURE WITH HIV-1 DRUG RESISTANCE

Variable[1]		NRTI Resistance Yes (N=64)	NRTI Resistance No (N=26)	P	NNRTI Resistance Yes (N=72)	NNRTI Resistance No (N=18)	P	Any Resistance Yes (N=73)	Any Resistance No (N=17)	P
Age at baseline, years Median [IQR], [N=90]		11 [8.0-14.0]	12.8 [11.0-14.0]	0.07	12.0 [9.0-14.0]	12.0 [9.0-13.0]	0.73	12.0 [9.0-14.0]	12.0 [10.0-13.0]	0.51
Sex, %Male, [N=90]		66%	54%	0.34	60%	72%	0.42	60%	71%	0.58
ART duration at baseline, months Median [IQR], [N=86]		49.5 [23.0-71.8]	25.5 [7.3-61.5]	0.11	**48.5 [23.0-72.0]**	**20.5 [4.8-51.8]**	**0.03**	48.0 [22.5-72.0]	24.0 [6.9-54.5]	0.08
WHO Clinical Stage at baseline, %Stage I, [N=90]		86%	81%	0.54	86%	79%	0.48	85%	82%	0.72
CD4 count at baseline, cells/mm^3 Median [IQR], [N=88]		481 [224-752]	483 [270-781]	0.79	481 [248-674]	584 [270-910]	0.50	482 [250-700]	485 [257-957]	0.66
CD4 count at ART initiation cells/mm^3, Median[IQR], [N=84]		280 [136-646]	265 [195-482]	0.71	245 [149-643]	292 [200-731]	0.44	261 [151-628]	270 [198-581]	0.67
Baseline Weight-for-age Z-score* Median[IQR], [N=89]		-1.5 [-2.1 – (-0.6)]	-1.7 [-2.2 – (-0.9)]	0.59	-1.5 [-2.1 – (-0.7)]	-1.8 [-2.4 – (-1.2)]	0.2	-1.5 [-2.1 – (-0.6)]	-1.8 [-2.6 – (-1.1)]	0.31
Baseline Height-for-age Z-score* Median[IQR], [N=89]		-1.3 [-2.0 – (-0.3)]	-1.6 [-2.3 – (-0.6)]	0.32	-1.3 [-2.1 – (-0.4)]	-1.5 [-2.1 – (-0.6)]	0.63	-1.3 [-2.1 – (-0.4)]	-1.4 [-2.1 – (-0.6)]	0.77
Baseline ART regimen [N=89]	%AZT-based$^{\psi}$	66%	64%	1.00	68%	56%	0.41	67%	59%	0.58
	%D4T-based$^{\psi}$	33%	20%	0.31	30%	28%	1.00	29%	29%	1.00
	%NVP-based	81%	71%	0.40	83%	60%	0.08	83%	60%	0.08
	%EFV-based	19%	29%	0.40	17%	40%	0.08	17%	40%	0.08
Drug substitution, %Yes, [N=84]		**57%**	**30%**	**0.04**	55%	27%	0.08	54%	29%	0.14
Adherence to ART, % sub-optimal$^{\chi}$, [N=90]		34%	27%	0.62	64%	17%	0.16	36%	18%	0.25

Table 2: Sociodemographic and clinical factors associated with NRTI, NNRTI, or any drug resistance. Participants with NNRTI resistance tended to have been on first-line cART longer than those without resistance (p=0.03), while a greater proportion of NRTI-resistant participants had undergone a drug substitution compared to those without NRTI resistance (p=0.04). Both trends hold for the other resistance groups.

AMPLIFICATION METHOD DOES NOT BIAS RESISTANCE MUTATION DETECTION

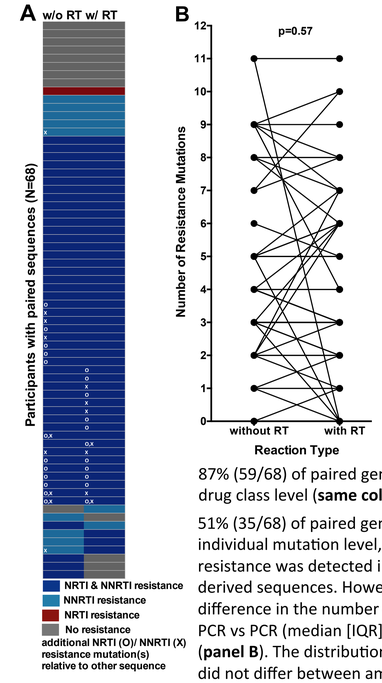

Figure 4: Concordance between resistance genotypes obtained with and without an initial Reverse Transcriptase step

(A) Resistance genotype concordance for participants for whom paired PCR and RT-PCR genotypes were available (one participant/ row), **(B)** Total number of resistance mutations observed in genotypes obtained with and without an initial RT step

N=68 (72%) participants had intact HIV-1 genotypes derived from each of PCR and RT-PCR (**panel A**)

87% (59/68) of paired genotypes were concordant at the drug class level (**same colour left vs right in panel A**)

51% (35/68) of paired genotypes were discordant at the individual mutation level, including four cases where resistance was detected in PCR-, but not RT-PCR-, derived sequences. However, there was no significant difference in the number of mutations detected by RT-PCR vs PCR (median [IQR] 3 [0-5] vs 3 [1-5], p=0.57) (**panel B**). The distribution of individual mutations also did not differ between amplification methods

REPLICATE GENOTYPING MAY INCREASE BREADTH OF DRUG RESISTANCE CAPTURED

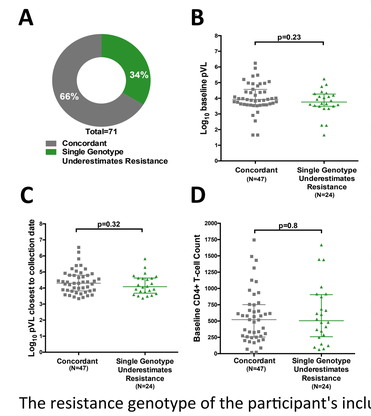

Figure 5. Impact of replicate genotyping on resistance interpretation
(A) concordant resistance genotypes at the individual drug level (grey) versus single genotype (green). **(B)** Log_{10} baseline plasma viral load for concordant (grey) versus discordant (green) genotypes at the individual drug level; **(C)** log_{10} plasma viral load at failure **(D)** CD4+ T-cell count at baseline.

N= 71 participants had >1 intact HIV genotype allowing for an assessment of the degree to which single genotyping may underestimate resistance.

The resistance genotype of the participant's inclusive consensus was compared to that of each individual sequence.

In 34% of participants (24/71) the inclusive consensus captured greater resistance at the individual drug level (**panel A**). However, no association between underestimation of drug resistance and Log_{10} baseline pVL, Log_{10} pVL closest to blood draw or baseline CD4+ T-cell count was observed (**panels B-D**).

CONCLUSIONS

A high level of HIV-1 drug resistance in Ethiopian children failing first-line cART was observed. Access to expanded expanded antiretroviral treatment options and implementation of routine and timely plasma viral load and drug resistance testing in Ethiopia and other resource-limited settings is urgently needed.