

# HIV Subtype D is Associated with Rapid CD4 Decline in Antiretroviral Therapy (ART)-Naïve Ugandan Children

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## Background

- Infection with HIV subtype D is associated with more rapid CD4 loss, shorter time to AIDS, and early death compared to HIV subtype A in studies of Kenyan and Ugandan adults
- There are no prospective data in children

## Objective

To determine if children infected with HIV subtype D experience more rapid CD4 cell count decline compared to children infected with HIV subtype A

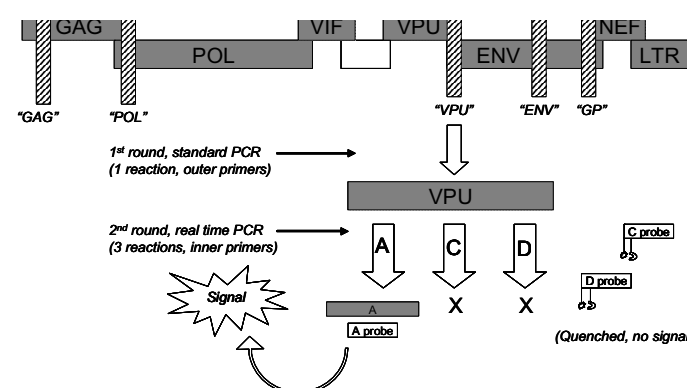
## Methods

### Subjects

- Children were enrolled from the Children with HIV and Malaria Project (CHAMP), an observational cohort in Kampala, Uganda.
- WHO clinical stage and CD4 status were assessed every 12 weeks.
- ART was initiated per age-specific Ugandan/WHO guidelines
- Children with at least 3 CD4 counts while ART-naïve were included
- If a child became eligible and started ART, data was censored thereafter

## Laboratory Techniques

- **CD4 cell count and %:** measured every 12 weeks (FACS Calibur, BD Biosciences)
- **Plasma HIV RNA:** fresh plasma samples tested every 12 weeks using Roche Amplicor (Version 1.5), with level of detection 400 copies/ml.
- **CD4 and CD8 T Cell Activation:** defined by % of cells with HLA-DR and CD38 co-expression from within 180 days of enrollment. From fresh peripheral blood mononuclear cells, a minimum of 30,000 CD3+ cells per sample were analyzed using a 4-color flow cytometer (FACS Calibur, BD Biosciences).
- **HIV Subtype:** banked frozen plasma samples were tested by Multi-region Hybridization Assay [Hoelscher et al., AIDS, 2002], a 2-stage, real-time, PCR-based technique that utilizes subtype-specific probes to identify HIV-subtype in 5 different genome regions (*env*, *gag*, *pol*, *vpu*, *gp-41*). The figure below models the technique for VPU region.

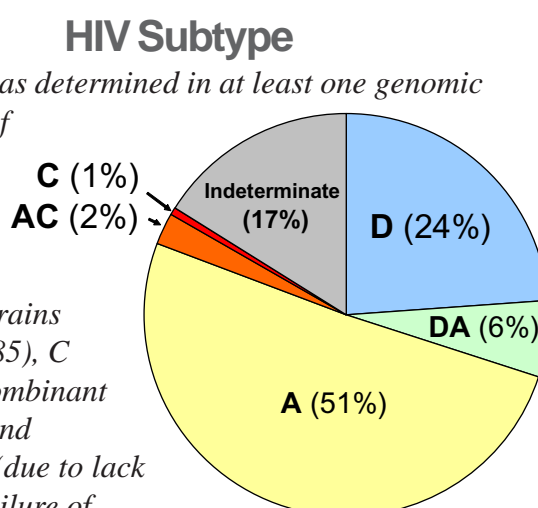


## Analysis

- The slope of CD4 change was modeled for each child using simple linear regression of all available CD4 counts (while ART-naïve).
- The Kruskal-Wallis equality-of-populations rank test, Cuzick test for trend, and simple and multivariate linear regression were used for analysis.

## Results

**HIV Subtype**  
HIV Subtype was determined in at least one genomic region in 140 of 168 children (83%), comprised of D (n=40), DA recombinant strains (n=10), A (n=85), C (n=1), AC recombinant strains (n=4) and indeterminate (due to lack of sample or failure of assay, n=28).



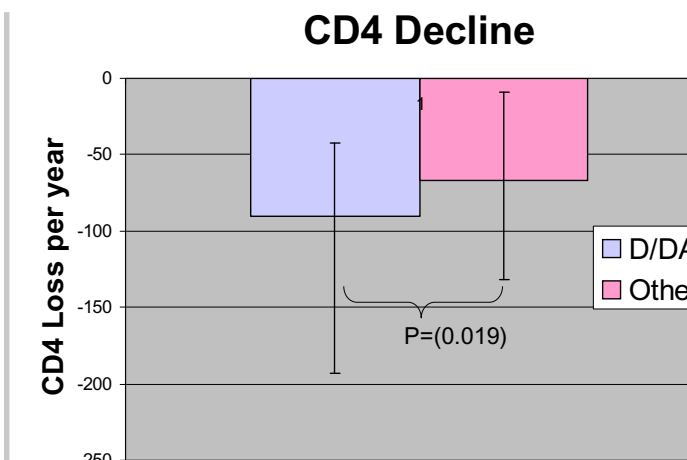
## Baseline Characteristics

	All Children	HIV Subtype Subgroups*	
		D or DA*	A, C, or AC*
Subjects, n (%)	168	50	90
Female, n (%)	99 (59%)	28 (56%)	56 (62%)
Age, years	6.4 (5.0-8.0)	6.0 (4.8-7.8)	6.5 (4.9-8.4)
CD4 Count (cells/μl)	802 (596-1116)	834 (587-1240)	748 (542-1056)
CD4%	26 (21-30)	25 (21-33)	25 (20-29)
HIV RNA (log <sub>10</sub> copies/ml)	4.9 (4.5-5.3)	4.9 (4.4-5.4)	5.1 (4.5-5.4)
WHO Stage I, n (%)	68 (40%)	18 (36%)	35 (39%)
II, n (%)	72 (43%)	27 (54%)	37 (41%)
III, n (%) <sup>o</sup>	28 (17%)	5 (10%)	18 (20%)
CD4+CD38+HLA-DR+ (%) <sup>†</sup>	11.6 (8-15)	11.7 (8-17)	12.3 (9-15)
CD8+CD38+HLA-DR+ (%) <sup>†</sup>	41.5 (32-50)	41.1 (33-51)	42.9 (33-51)

**NOTES:** All values are median (interquartile range) unless otherwise specified.  
\* HIV Subtype D in one or more regions, includes DA recombinant strains.  
~ A, C, or AC recombinant strains. In 28 cases subtype was not determined.  
† T-Cell activation was evaluated in 125 (74%) cases, 35 were D or DA and 67 were A, C, or AC recombinant strains.  
<sup>o</sup> Certain WHO stage III criteria are not indications for ART initiation, including pulmonary tuberculosis, lymphoid interstitial pneumonitis, oral hairy leukoplakia, thrombocytopenia.  
<sup>^</sup> There were no statistically significant differences between the subtype subgroups in any baseline measures.

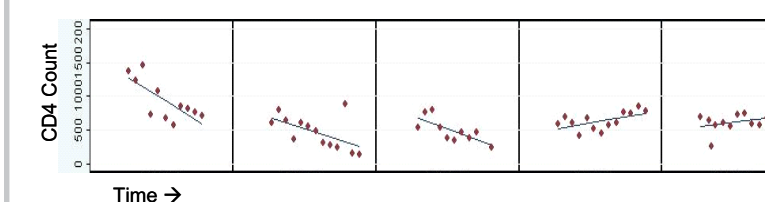
## CD4 Decline

- A median 16 CD4 count values per child (range 4–13) over a median 1007 days (range 167–1108) were analyzed.
- HIV Subtype D/DA (D and D-A recombinant) strains were associated with a mean CD4 decline of 172 vs. 72 cells/year for non-D-containing strains (p=0.019) in univariate analysis.
  - Young age (p<0.001) and high baseline CD4 (p<0.001) were also associated with CD4 decline.
  - Baseline CD4% (p=0.11), being WHO clinical stage III (p=0.07), plasma HIV RNA (p=0.62), CD4 (p=0.79) and CD8 activation (p=0.60), WHO stage at enrollment (p=0.18) and gender (p=0.99) were not.



D/DA: HIV Subtype D and DA recombinant strains.  
Other: A, C, and CA recombinants. Values are medians, +/- IQR. P value is for Kruskal-Wallis comparison.

## Example Slopes



## Predictors of CD4 Slope\*

Variable	Coef.	P value	95% Conf.
Subtype D/DA	-0.183	0.03	-0.4 to -0.1
WHO Stage III	0.232	0.04	0.01 to 0.45
High CD4 count	-0.0005	<0.001	-0.0007 to -0.0003
Age	-0.022	0.29	-0.02 to 0.06
Intercept	0.036	0.29	-0.02 to 0.06

\*Multivariate linear regression

## Limitations

- Children with lower viral loads are more likely to have indeterminate subtype testing, which could have altered overall subtype results.
- Linear regression analysis is vulnerable to effects of outlying values.

## Discussion

- In ART-naïve Ugandan children >1 year of age, HIV subtype D was associated with more rapid CD4 decline compared to non-D strains.
- The increased pathogenicity of HIV subtype D is not explained by differences in baseline CD4 count, CD4%, HIV RNA, or CD4 or CD8 activation.
- Further study of recombinant strains and the association of subtype with CXCR4/CCR5 coreceptor tropism may yield insight into this subtype-specific increased pathogenicity.

## Acknowledgments

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