



The Impact of Age, Sex, and in utero Exposure to Antiretroviral Therapy on Relative LINE-1 Content in Infants Born to Mothers Living with HIV

Michael Afe^{1,2}, Izabella Gadawsk², Helene C.F. Cote^{2,3,4}, and the Children and Women: AntiRetroviral therapy and Markers of Aging (CARMA) team.

¹University of Jena, Germany, ²Department of Pathology and Laboratory Medicine, ³Centre for Blood Research, ⁴Edwin S.H. Leong Healthy Aging Program, University of British Columbia.



Contact: afemike@student.ubc.ca

Background

- Long Interspersed Nuclear Element-1 (LINE-1) is an autonomous transposable element whose activity has been linked to genetic diversity during embryonic development and genome instability and diseases later in life.

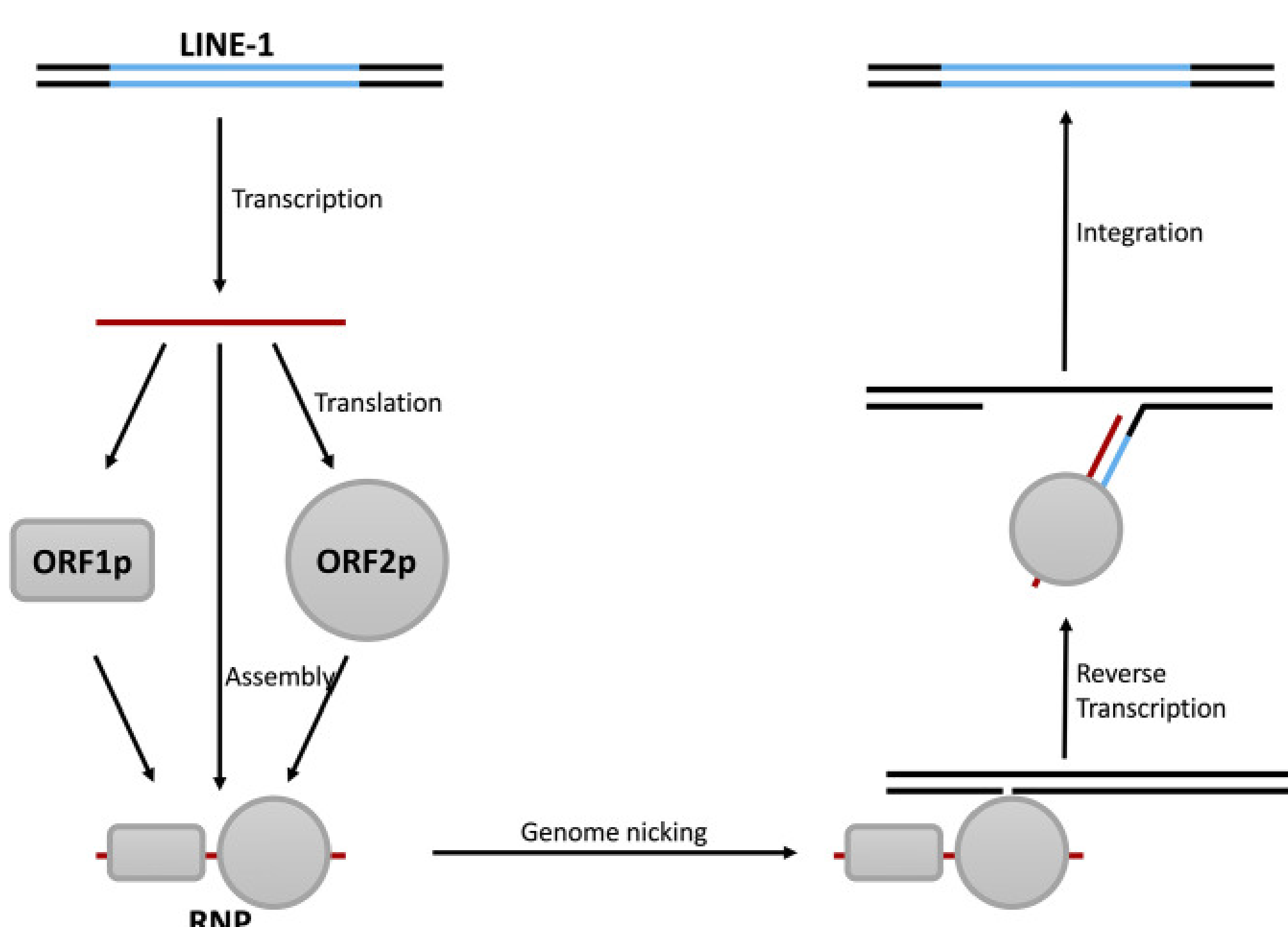


Figure 1: Steps of LINE-1 retrotransposition. The replication of LINE-1 starts with the transcription of LINE-1 RNA and is followed by the synthesis of the LINE-1 proteins ORF1p and ORF2p. ORF1p, ORF2p, and LINE-1 RNA then form LINE-1 RNPs with the help of other cellular proteins. LINE-1 RNPs then bind to genomic DNA and induce nicks with the endonuclease activity of ORF2p. This results in the unwinding of the DNA and leaves a protruding single-stranded fragment as the primer, and LINE-1 cDNA is synthesized with the reverse transcriptase activity of ORF2p. Finally, through a series of unknown mechanism(s), the double-stranded form of LINE-1 DNA is synthesized and integrated into the genome.

- Combination antiretroviral therapy (cART) is a widely used treatment for HIV that effectively suppresses viral replication. cART may also regulate LINE-1 activity.
- Intrinsic biological variables such as **sex** and **age** may also play a role in the interaction between cART and LINE-1.

Objectives

To investigate the relationship between age, sex, and in utero cART exposure on LINE-1 content in infants born to mothers with HIV.

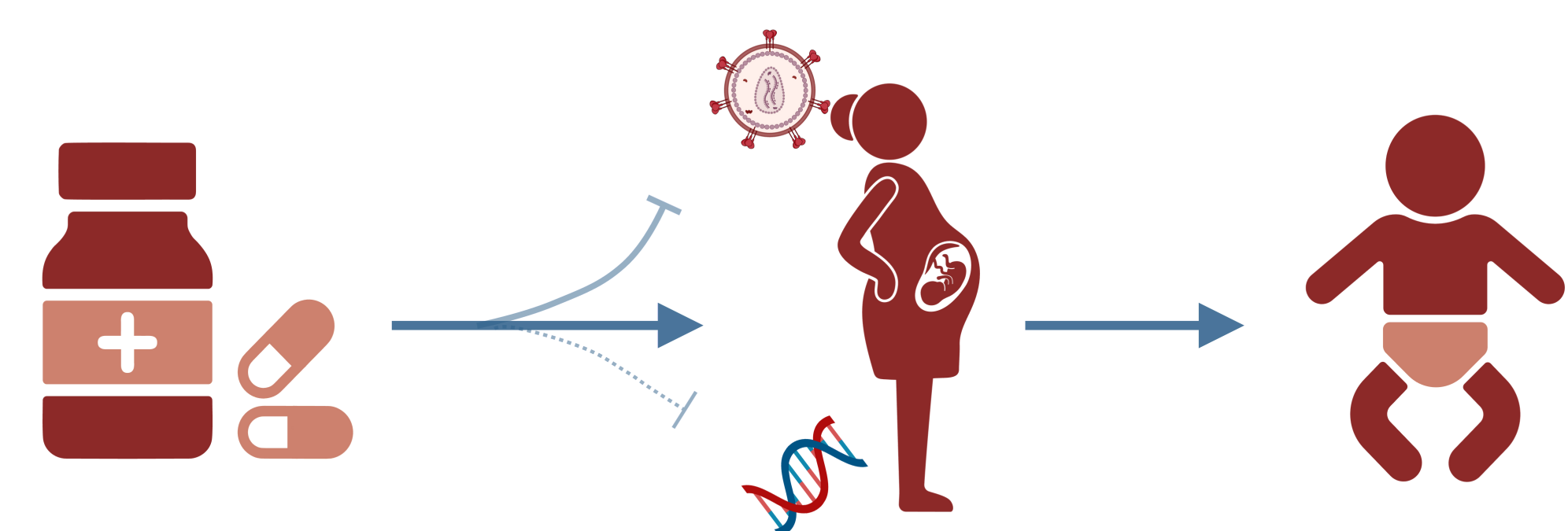


Figure 2: Diagrammatic Overview of Antiretroviral Therapy Influence on LINE-1 Activity from Maternal Treatment to Infant Outcomes. The illustration shows antiretroviral therapy (ARV) administration and its dual role in HIV inhibition and potential modulation of LINE-1 activity. The solid blue line represents the established effect of ARVs in suppressing HIV replication, while the dotted blue line suggests the possible influence of ARVs on LINE-1 activity within the DNA. The pathway is depicted flowing from the mother, through in utero exposure, to the infant, highlighting the direct transference and its implications for LINE-1 content in early development.

Hypothesis

The Relative LINE-1 content in infants is significantly influenced by in utero exposure to combination Antiretroviral Therapy (cART), with variations attributable to the infants' age and sex.

Methodology

- LINE-1 content was quantified by monochrome multiplex qPCR (MMqPCR) in **85 children** enrolled in the CARMA cohort.
- 28** Children exposed and uninfected (**CHEU-1**), **29** Children exposed from the second trimester onward (**CHEU-2**), and **28** Children unexposed and uninfected (**CHUU**).
- MMqPCR results were investigated through Univariate and Multivariate analysis.

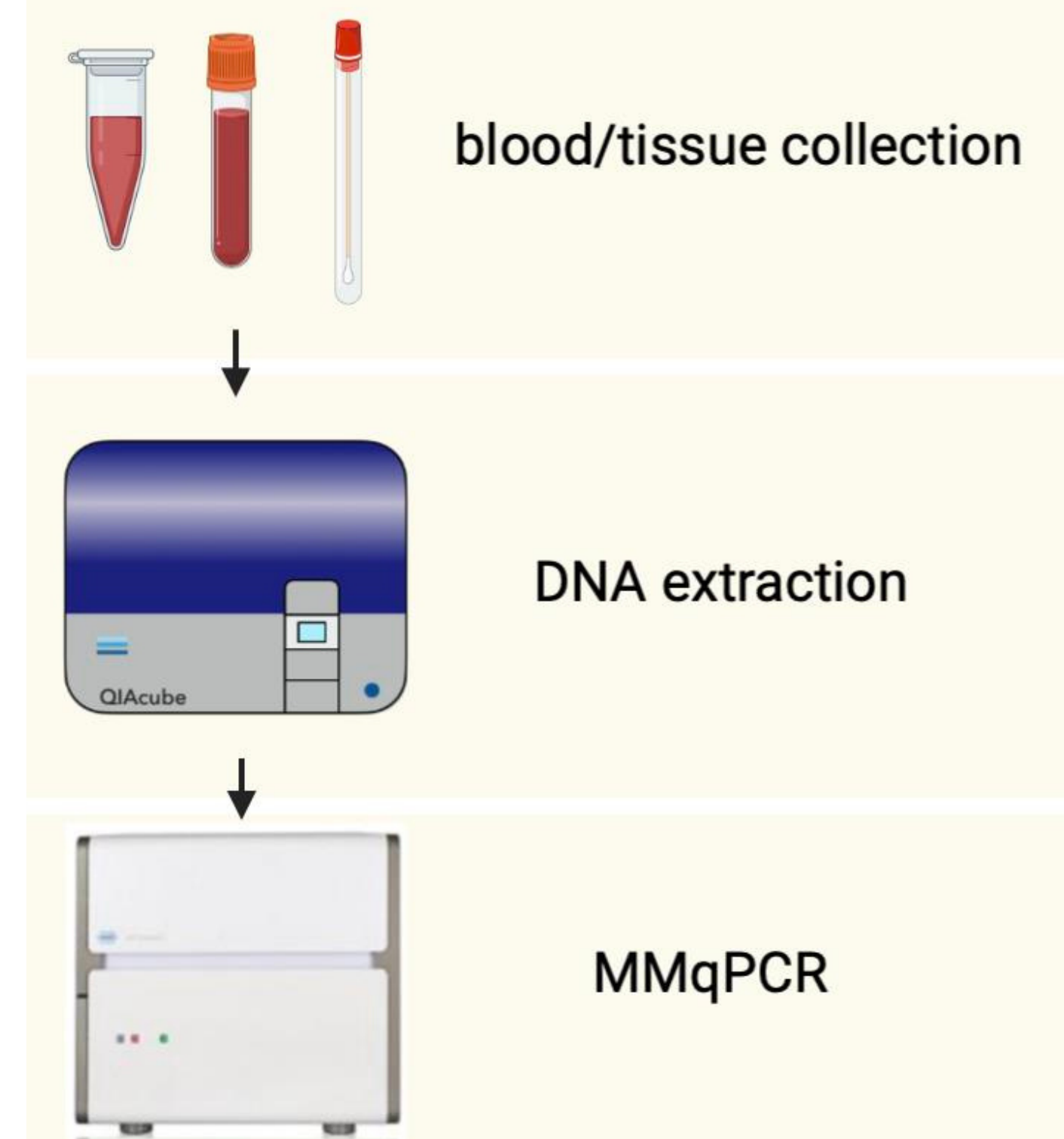


Figure 3: Diagram of MMqPCR measurement for infant whole blood. Whole blood genomic DNA was extracted on a Qiacube machine using Qiagen kits. MMqPCR was done on a Roche Lightcycler 480

Table 1. Demographic information of participants in the dataset

| Exposure | Total Participants | Mean Age (Years) | Female Count | Male Count |
|----------|--------------------|------------------|--------------|------------|
| CHEU-1 | 28 | 1.24 | 13 | 15 |
| CHEU-2 | 29 | 1.32 | 15 | 14 |
| CHUU | 28 | 1.31 | 15 | 13 |

Results

Univariate Analysis:

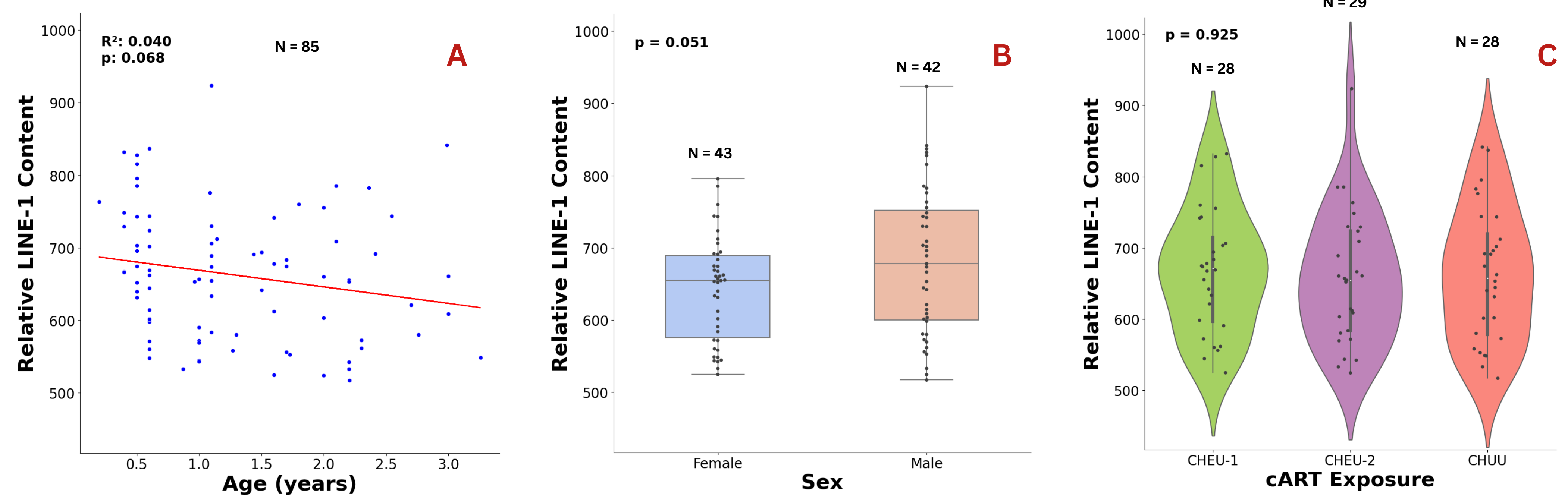


Figure 4: Univariate Analysis of LINE-1 Content in Infants with HIV Exposure

A Linear regression: illustrates the variation in relative LINE-1 content across different ages in years, highlighting age-related trends. **B** Boxplot: compares LINE-1 content between male and female infants, showing potential sex-based differences. T-test pvalue is indicated. **C** Violin plot: shows the impact of in utero exposure to combination Antiretroviral Therapy (cART), categorizing infants based on exposure timing—CHEU-1, CHEU-2, and CHUU. Anova pvalue is indicated.

Multivariate Analysis:

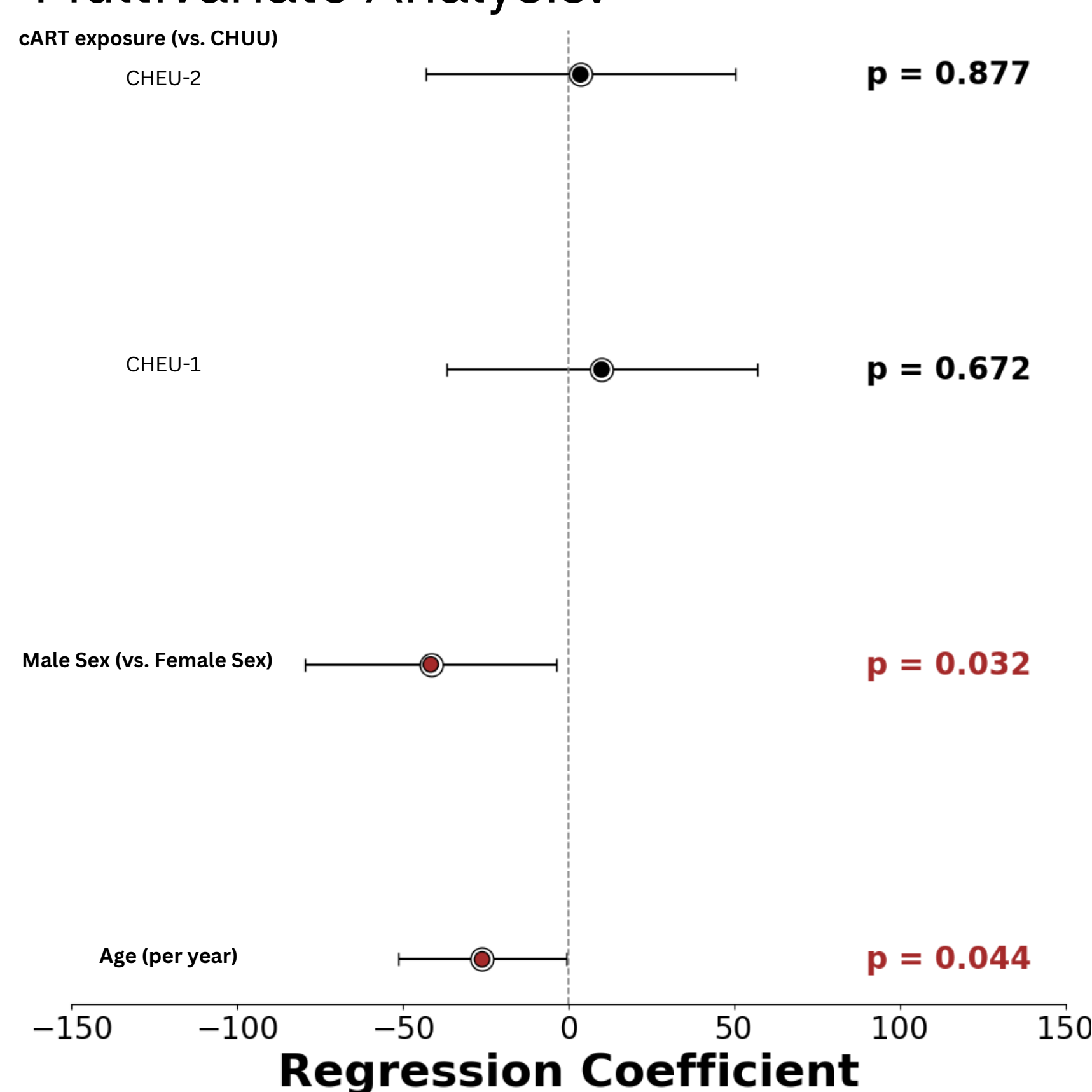


Figure 5: Forest Plot of Multivariable Linear Regression Analysis Assessing Factors Influencing LINE-1 Content in Infants

This plot visualizes the results of a multivariable linear regression model examining the influence of age, sex, and different in utero cART exposure categories on Relative LINE-1 content in infants. Each line in the plot represents a variable, displaying its regression coefficient along with 95% confidence intervals. The color of the scatter points indicates statistical significance, with brown denoting $p < 0.05$

Conclusion

Our findings seem to align with LINE-1 being active in early development. Furthermore, the observation that females have higher relative LINE-1 content could be related to sex-specific differences, such as epigenetic modifications that contribute to differential gene expression and could influence LINE-1 content.

Acknowledgements

We thank the women (and infants) who participated in our study, our CARMA collaborators, and the members of the Côté lab for their support

