Exploring Placenta Mitochondrial DNA Mutation Burden and Pregnancy Risk Factors in Women with HIV: preliminary findings from a molecular barcoding approach

Rachel A Dunn[1,2,3], Evelyn Maan[3], Chelsea Elwood[4], Deborah Money[3,4], Hélène CF Côté[1,2,3], and the CIHR Team in Cellular Aging and HIV Comorbidities in Women and Children (CARMA-PREG)

[1]Department of Pathology & Laboratory Medicine;
[2]Centre for Blood Research;
[3]Women's Health Research Institute,
[4]Department of Obstetrics and Gynecology,
all at University of British Columbia, Vancouver, BC, Canada

Introduction

- ~18 million women are living with HIV, most of whom are of reproductive age
- At 3X higher risk for preterm birth (PTB)
- Upon HIV infection, mitochondrial DNA copy number per cell (mtDNA content) decreases
- mtDNA content increases when individuals are on certain classes of antiretrovirals (ARVs)
- These fluctuations could lead to clonal amplification of existing mtDNA mutations

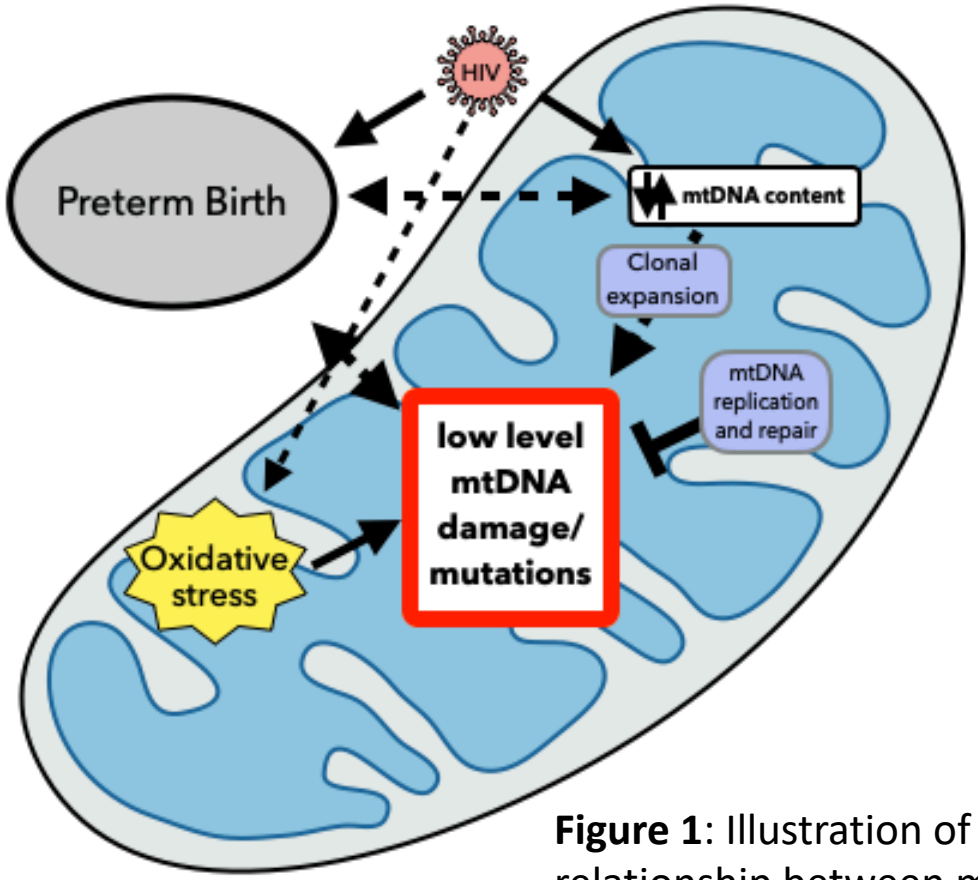


Figure 1: Illustration of relationship between mtDNA mutations, aging and HIV

Hypotheses:

1. Pregnancy adverse outcome risk factors including smoking, HIV infection, and/or maternal age are associated with a higher rate of placenta mtDNA mutations
2. Elevated placenta mtDNA mutation burden is associated with PT delivery

Cohort Design

Table 1: CARMA cohort placenta biospecimens selection for this exploratory study (n=64), all values presented as n (%) or mean (SD).

	Living with HIV (HIV+) n = 37	Not living with HIV (HIV-) n = 27
Delivery		
Term delivery	24 (65)	17 (63)
Preterm delivery (<37w)	13 (35)	10 (37)
Smoking		
Never in pregnancy	27 (73)	15 (56)
Quit in pregnancy	2 (5)	1 (4)
Throughout pregnancy	7 (19)	6 (22)
Unknown	1 (3)	5 (19)
Maternal Age (y)	32.4 (5.1)	31.0 (4.2)

Study Methodology

64 placenta specimens

↓

DNA extraction, mtDNA labelling with primer ID

↓

mtDNA purification and amplification

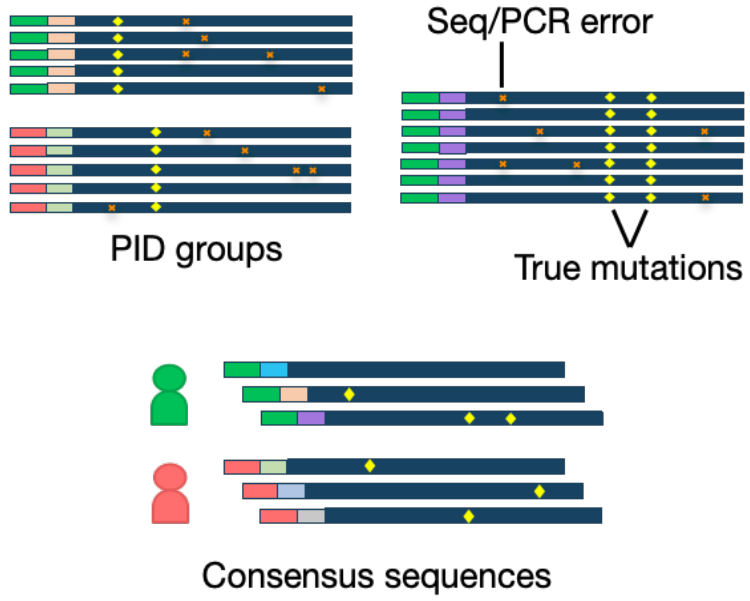
↓

Illumina MiSeq 2X300 read sequencing

↓

Primer ID-based read alignment → Filtering out sequencing and PCR errors → Identification of rare mutations

Figure 2: Bioinformatics pipeline schematic to reassemble reads and identify true mutations while filtering out PCR and sequencing errors.



Results

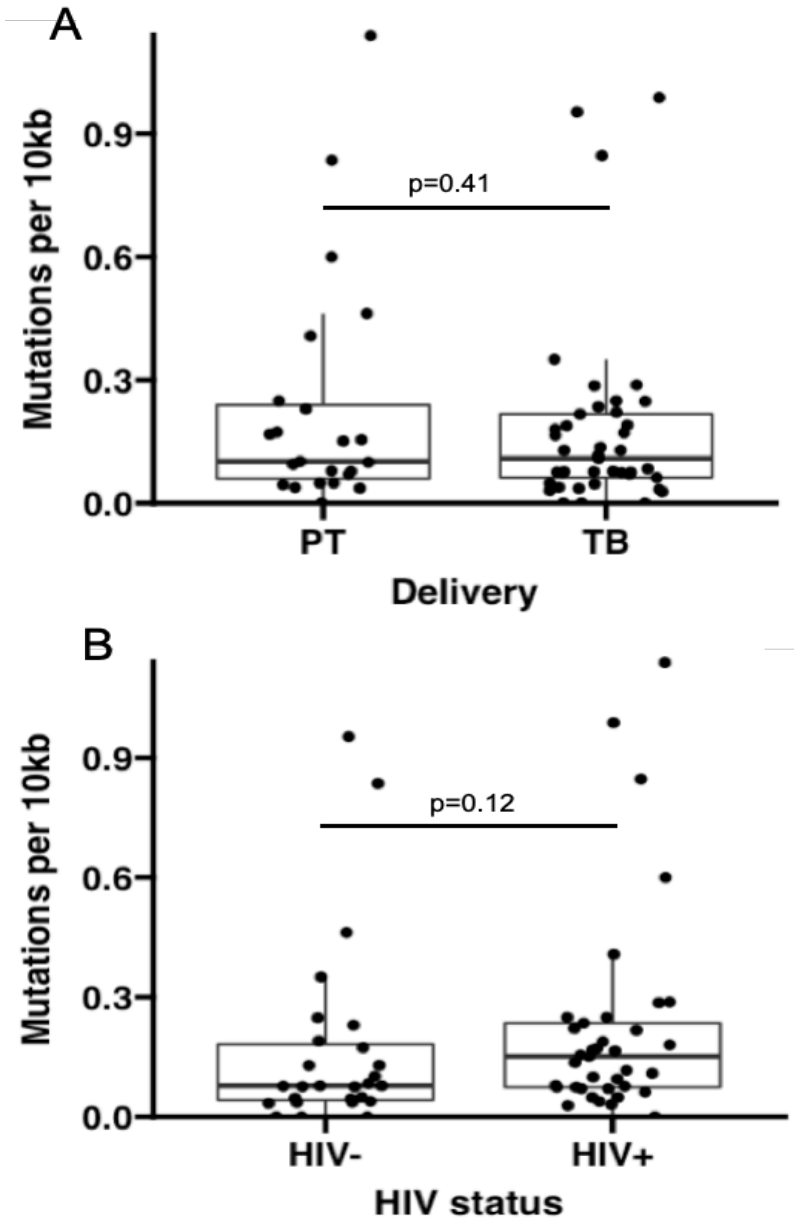


Figure 4: Placenta mtDNA mutations shows a weak trend toward an association with HIV (A) but no association is detected with PT delivery (B), mutations that are present with a frequency of >2% are excluded from this analysis (Mann Whitney-U test)

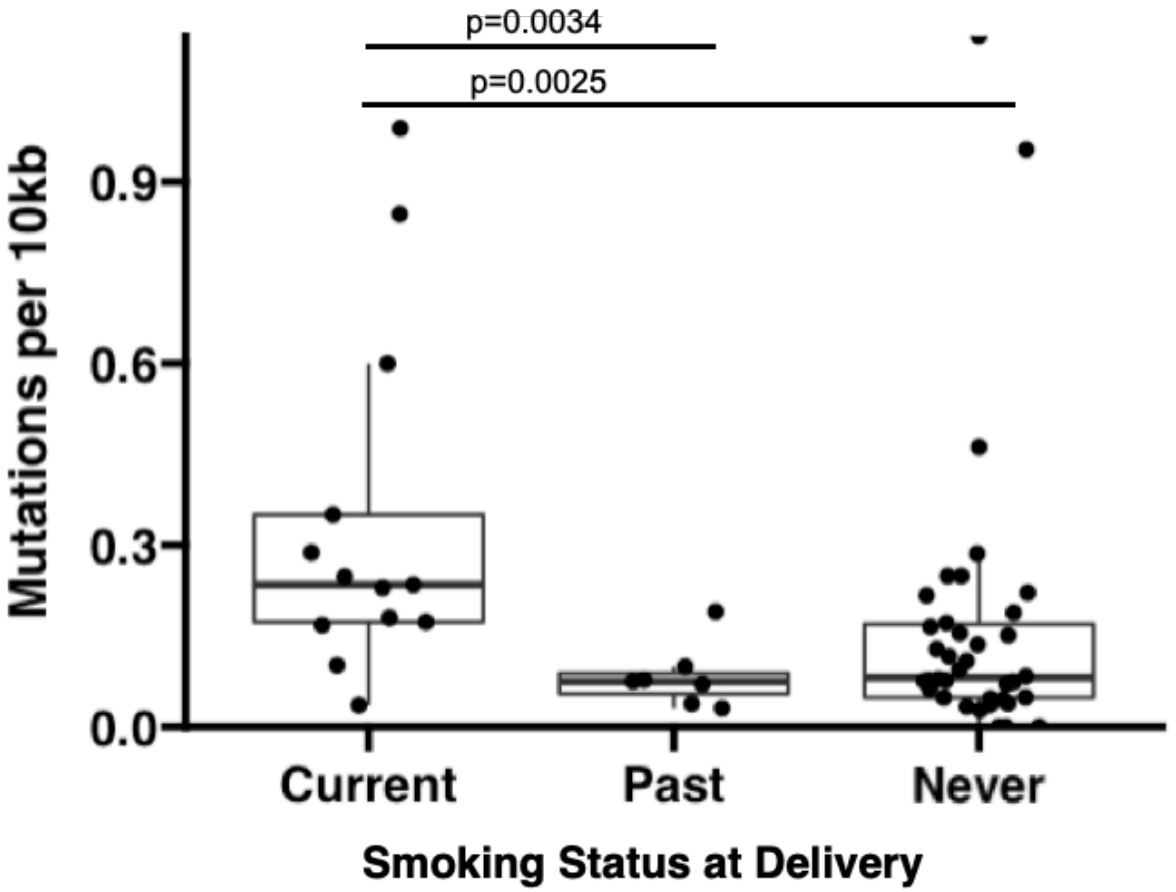


Figure 3: Elevated placenta mtDNA mutations shows a significant association with smoking for the duration of pregnancy, compared to those who smoked before or early in pregnancy but stopped during pregnancy (Mann Whitney-U test)

Results

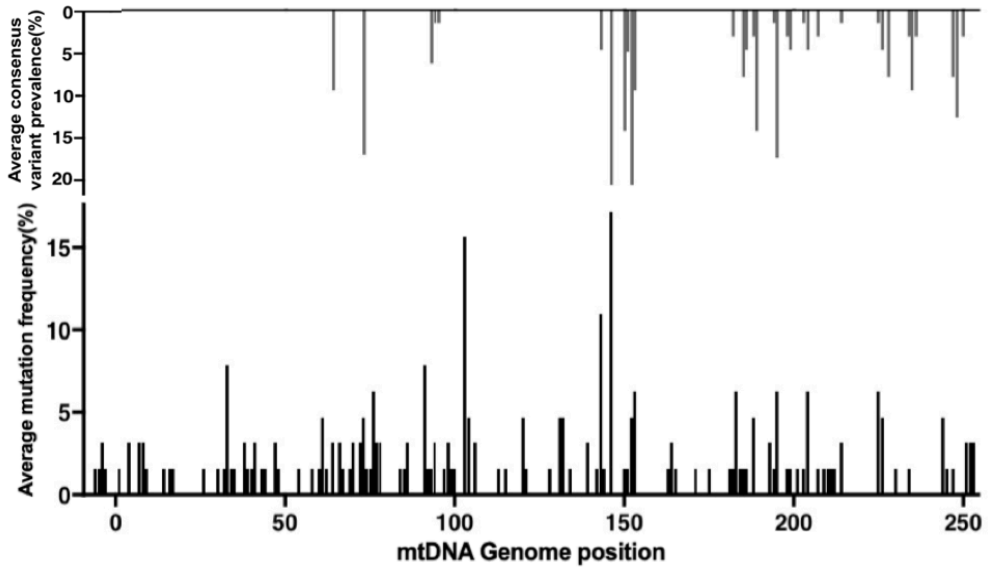
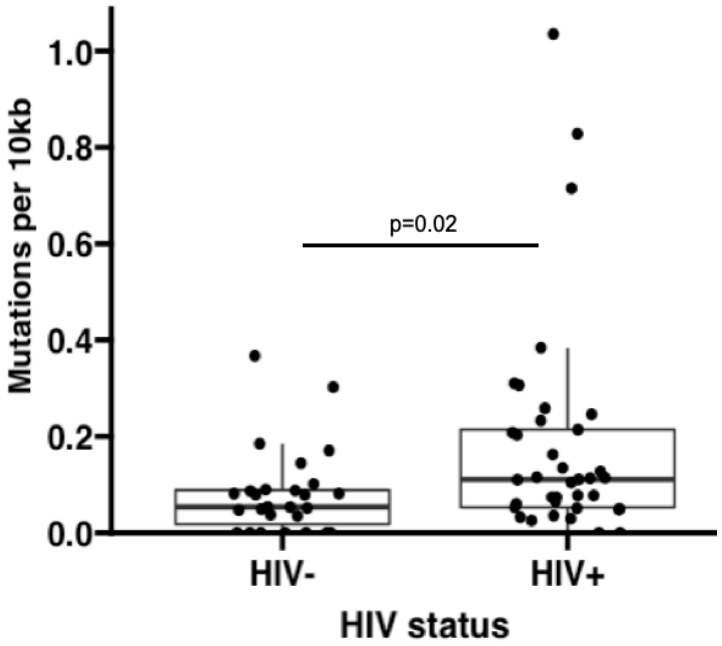


Figure 5: **A)** Prevalence of variants among consensus sequences of all assayed specimens (grey). **B)** Percentage of specimens with mutations (black) across the interrogated region of mitochondrial genome (MT16559- MT254). Majority of mutations are somatic (present in <2% of molecules). Several positions that have a high prevalence of mutations are also seen to have variants among specimens we sequenced (observed in ⩾10% of specimens)

Figure 6: Subanalysis of placenta mtDNA mutations at 252 conserved positions (with no homoplasmic variants in our sample population). HIV+ status is significantly associated with elevated mutation burden in these conserved regions



Conclusions

- Living with HIV shows a trend towards higher levels of placenta mtDNA mutations, this may become clear with a larger sample size
- No significant association detected between preterm birth and placenta mtDNA mutations
- Smoking up until the time of delivery is associated with elevated mtDNA mutation burden
- The placenta mtDNA mutation burden among WLWH appears increased at highly conserved positions along the mtDNA genome

Future directions: study of >300 participants, investigating cART, and other pregnancy complications