Placental and blood mitochondrial DNA heteroplasmies in women living with HIV and their association with preterm birth

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BACKGROUND

20.2 million women and girls worldwide live with HIV

Most women are of childbearing age

Women living with HIV are **2.66 times** more likely to deliver preterm

WLWH experience elevated rates of mitochondrial disease and mitochondrial DNA damage

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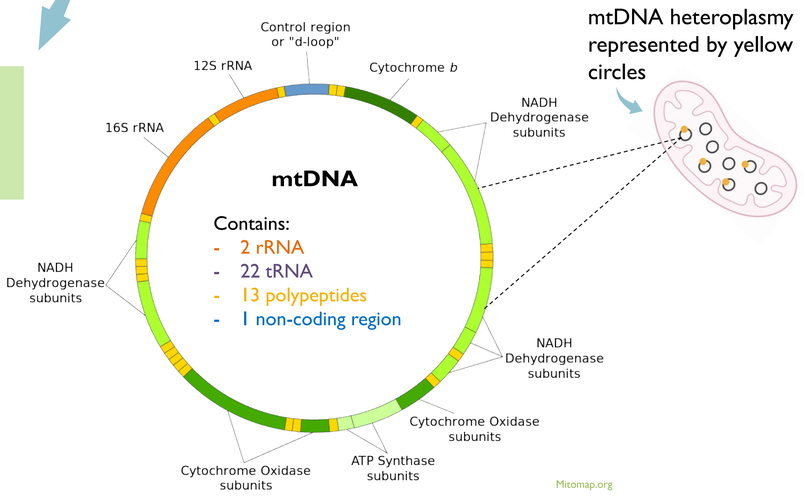
mtDNA heteroplasmies are mutations with a frequency ≥2%

Preterm birth is:
- birth at <37 weeks of gestation
- associated with both short and long-term negative health outcomes



OBJECTIVES

- To investigate the **mitochondrial DNA heteroplasmies** present in the placental tissue of a cohort of **women living with and without HIV** and determine any association with **preterm birth status**.
- To determine if previously-identified placental pathogenic mtDNA heteroplasmies are present within **other fetal tissues and maternal blood**.

HYPOTHESES

- A **greater** number of pathogenic mtDNA heteroplasmies will be associated with **HIV-positive** and **preterm** birth status.
- Pathogenic mtDNA heteroplasmies present in placental tissue will also be present in maternal blood and tissues.

METHODS

Sequenced a total of 69 samples with participants from two different study groups:
- **C**hildren and Women: **A**ntiretrovirals and **M**arkers of **A**ging (CARMA)-PREG
- **E**pigenetics of **P**lacenta **i**n **C**omplications of Pregnancy (EPIC)

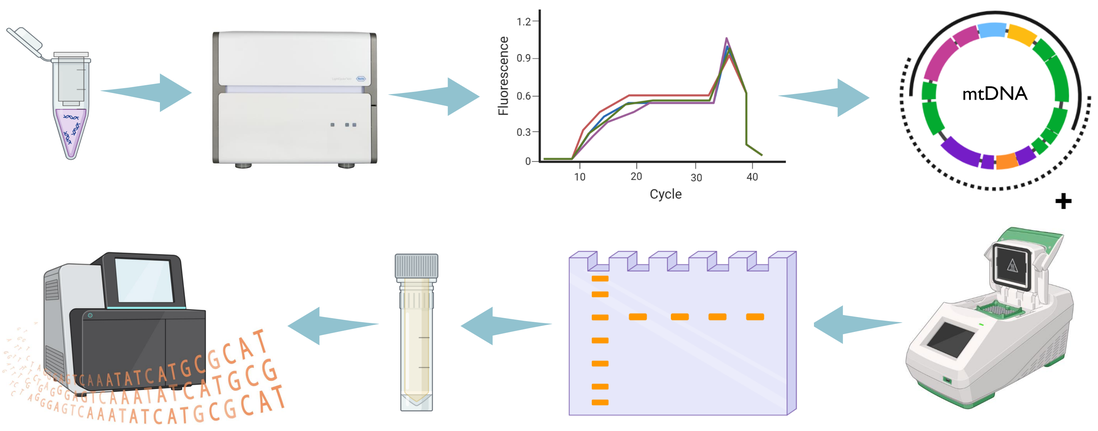


Fig 1. Workflow for mitochondrial whole-genome sequencing (mtWGS): DNA extraction (QIAamp Mini Kit), mtDNA qPCR (LightCycler480), long PCR, gel extraction, mtDNA sequencing (Illumina MiSeq), and sequence processing (not pictured)

	Term (n = 29)	Preterm (n = 32)	p-value
Maternal HIV Status, n (%)			1.0000
Living with HIV	12 (41.4)	13 (40.6)	
Not living with HIV	17 (58.6)	19 (59.4)	
Maternal Ethnicity, n (%)			0.1171
African/Caribbean/Black	1 (3.45)	6 (18.6)	
Indigenous	7 (24.1)	5 (15.6)	
White/European	14 (48.3)	8 (25.0)	
Other	1 (3.45)	2 (6.25)	
Missing	6 (20.7)	11 (34.4)	
Delivery Type, n (%)			**0.0096**
Vaginal	23 (79.3)	13 (40.6)	
Elective C-section	4 (13.8)	14 (43.8)	
Emergency C-section	2 (6.90)	5 (15.6)	
Preeclampsia, n (%)			**0.0091**
Yes	3 (10.3)	13 (40.6)	
No	26 (89.7)	19 (59.4)	
Maternal Age (years), median [IQR] (range)	33.4 [7.20] (21.6, 40.4)	33.8 [7.55] (19.7, 42.9)	0.3515
Gestational Age at Birth (weeks), median [IQR] (range)	39.3 [2.10] (37.1, 42.1)	35.2 [2.55] (24.1, 36.9)	**<0.0001**

Fig 2. Demographics of study participants. The only significant variables are delivery type, preeclampsia, and gestational age at birth.

RESULTS

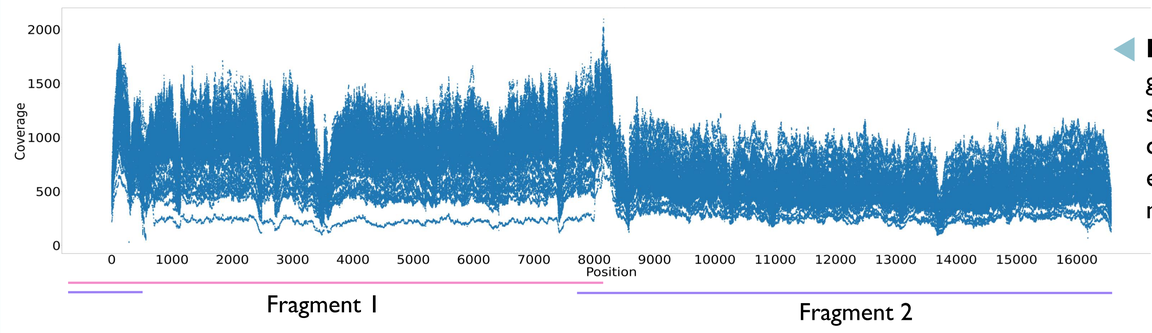


Fig 3. Next generation sequencing coverage for each base in the mtDNA

Fragment 1 reads are generally higher than Fragment 2, but reads are variable by sample.

Adjusting read trimming and alignment has removed low coverage areas and raised overall coverage.

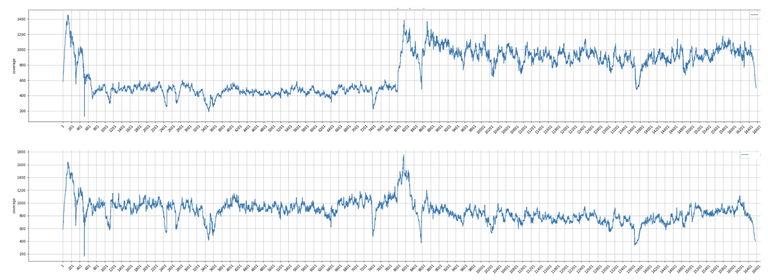
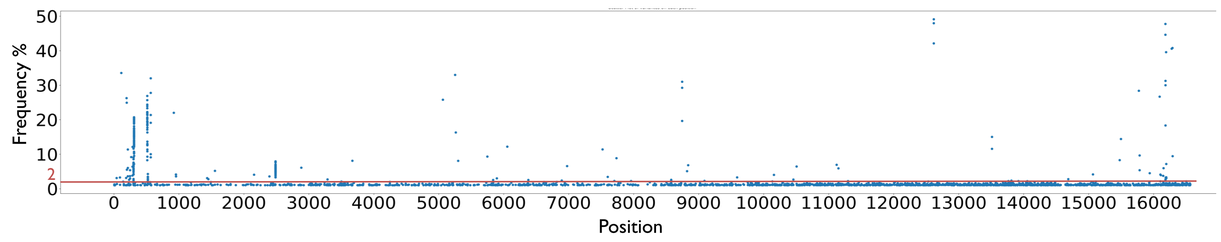


Fig 4. Heteroplasmy at each position in the mtDNA



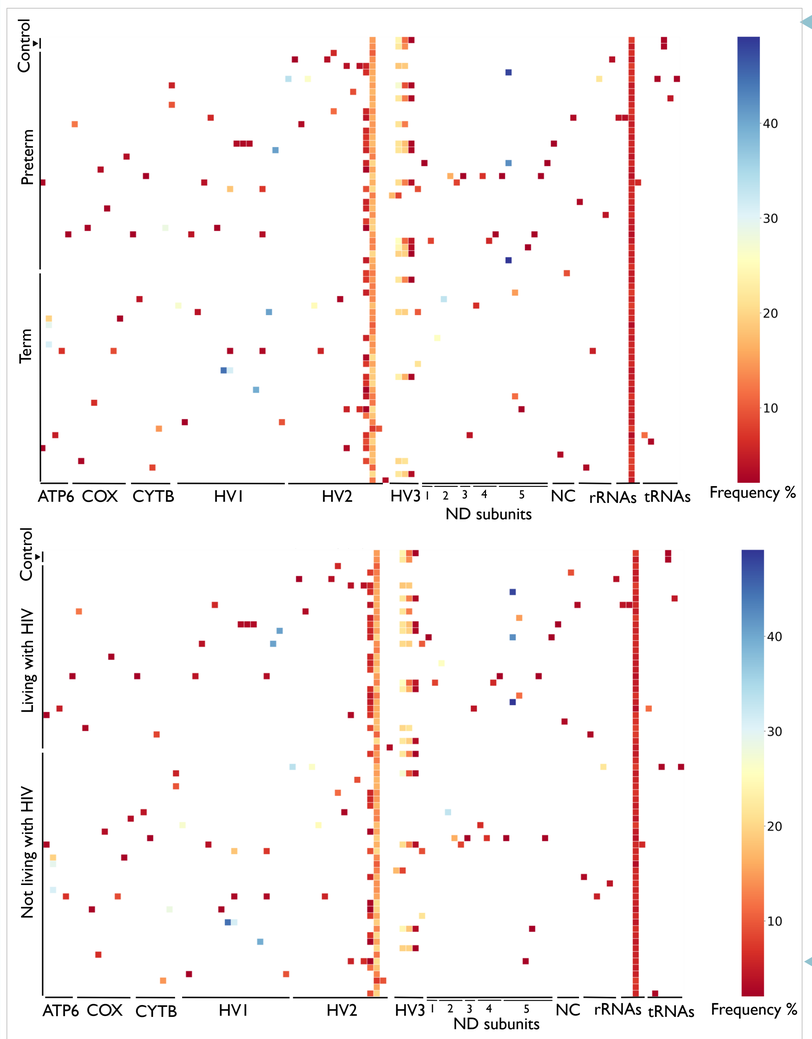


Fig 5a. Gene-specific heteroplasmy frequency separated by preterm and term birth groups. COX: Cytochrome c oxidase subunits; CYTB: Cytochrome b; HV1: hypervariable region 1 ND: NADH Dehydrogenase; NC: non-coding region

Position 310 (HV2) is a **non-pathogenic haplogroup marker.** Levels of heteroplasmy vary from 10% to 95% with nearly every sample having some level of heteroplasmy.

Position 2487 (rRNA) is an artifact of alignment. The aforementioned **new alignment method removes heteroplasmy as this location**, while significant pathogenic heteroplasmies remain.

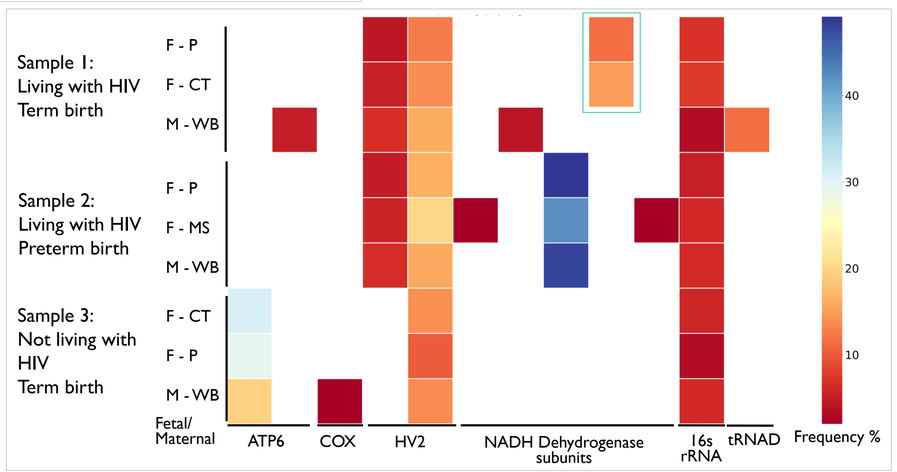
Preterm and term groups differ the most in the HV3 and NADH Dehydrogenase subunit areas.

Living with HIV and not living with HIV groups do not visually differ in heteroplasmy location.

Fig 5b. Gene-specific heteroplasmy frequency separated by participants living with HIV and participants not living with HIV

The mutation in the teal box (G13513A) is **confirmed pathogenic** for Leigh Disease/MELAS/LHON-MELAS overlap syndrome. Interestingly, the mutation is **present in both fetal tissues** at very similar levels (15% and 11.5%) but is **not present in maternal blood**.

Fig 6. Multi-tissue comparison of heteroplasmies between fetal and maternal tissues. P: placenta; CT: cord tissue; MS: mouth swab; WB: whole blood.



SIGNIFICANCE & FUTURE DIRECTIONS

This study will contribute vital information towards understanding of the role of mitochondrial health in pregnancy. It will also support the current body of research on the differences in mitochondrial health experienced by people living with HIV.

ACKNOWLEDGEMENTS

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