Low frequency mitochondrial DNA mutation hotspots in blood and placenta

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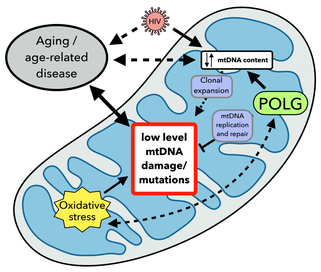
Introduction

- Mitochondrial genetics is challenging due to:
  - Heterogeneity
  - High copy number (10^2 - 10^4)
  - High turnover rate
  - Lower fidelity of polymerase gamma (POLG) which is prone to introducing transition mutations (A↔G, C↔T)
- Deep sequencing is unreliable for detection of low-level mutations (<2%)
- Both HIV and elevated mtDNA mutations are associated with aging and age related diseases
- Mitochondrial D-loop is a hypervariable, non-coding region containing one of the origins of replication

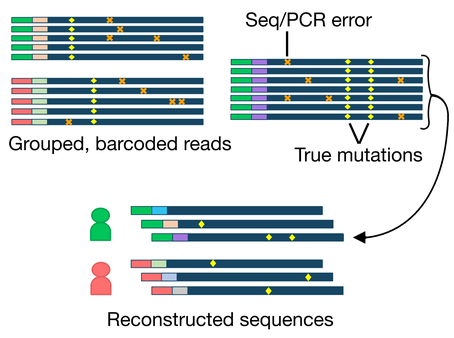


▶ **Figure 1:** Illustration of relationship between mtDNA mutations, aging and HIV

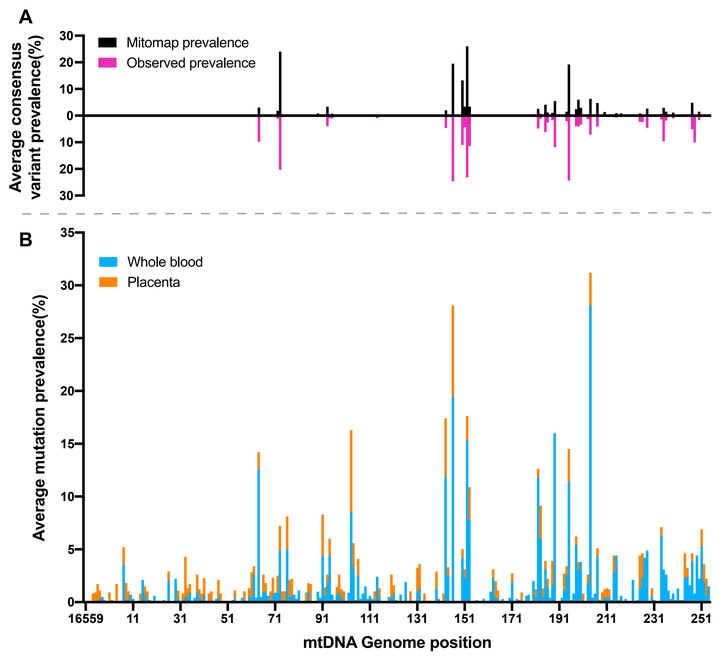
Study Methodology

544 Specimens (480 whole blood, 64 placenta)
↓
DNA extraction & mtDNA barcoding
↓
Amplicon purification & amplification
↓
Illumina MiSeq 2x300bp Sequencing
↓
Primer ID-based read alignment → Library preparation artifact removal → Identification of rare mutations

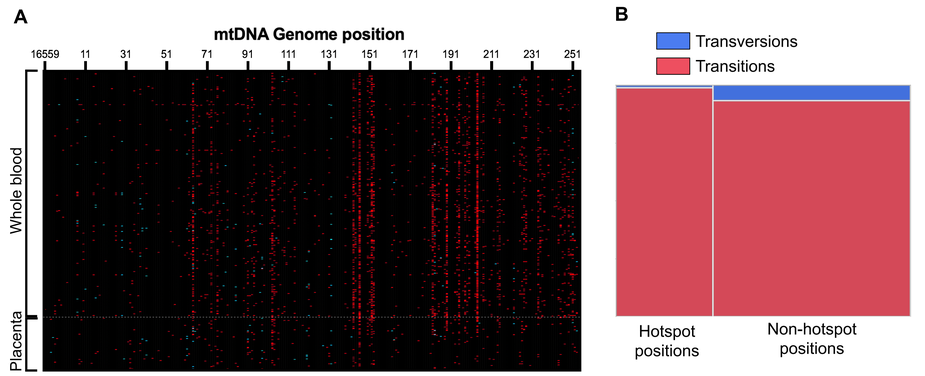
▼ **Figure 2**: Bioinformatics pipeline schematic to reassemble reads and identify true mutations while filtering out PCR and sequencing errors.



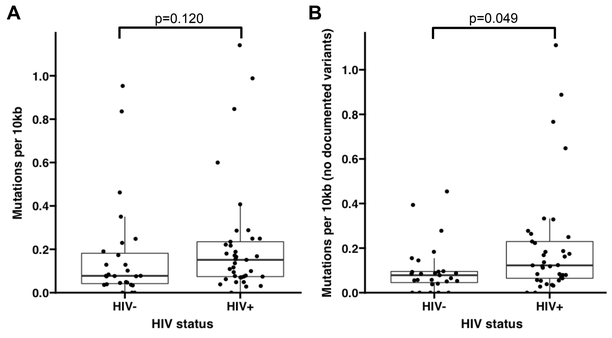
Results



▲ **Figure 3**: **A)** Prevalence of documented variants reported in MITOMAP, curated from mtDNA sequences in GenBank (black) is similar to that seen among the consensus sequences of study specimen (pink). **B)** Average prevalence of mutations among specimens in whole blood (blue) and placenta (orange) across the interrogated region of mitochondrial genome (MT16559-MT254). **Majority of mutations are somatic (present in <2% of molecules). Overall mutation rate is higher, and hotspots (observed in ⩾10% of specimens) are more pronounced in whole blood compared to placenta. Conserved regions among consensus sequences of individuals appear to contain less mutations.**



▲ **Figure 4**: **A)** Heatmap showing transition and transversion mutations (A↔C, A↔T, C↔G, G↔T) — red and blue respectively — for all assayed specimens. Hotspots for transitions are evident, especially in whole blood. **B)** Mosaic plot illustrating the **proportion of transition mutation is significantly higher among hotspot positions compared to non-hotspot positions (98.9% vs 92.9% of all mutations) (Fisher's exact test; p<0.0001).**



▶ **Figure 5**: **A)** Total mutation burden (mutation per 10,000bp) among placenta specimens of people living with HIV (HIV+) and uninfected individuals (HIV-) among all positions.
B) Subanalysis of 234 conserved positions (with no documented variants in MITOMAP). **HIV+ status is significantly associated with elevated mutation burden in conserved regions (Mann Whitney-U test)**

Conclusions

- Positions on the mitochondrial genome that are more tolerant to variants have a greater prevalence of rare mutations observed as hotspots, in both peripheral whole blood and placenta tissue **(Fig. 3)**
- Among the highly variable positions, transition mutations are significantly more prevalent compared to transversion mutations, suggesting polymerase gamma errors may be a driver in these mutation hotspots **(Fig. 4)**
- In placenta tissue, HIV infection is associated with an higher total mutation burden among conserved positions of the mtDNA Dloop **(Fig. 5B)**
- Together these finding may provide some insight on the link between mtDNA mutations, HIV and aging

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