Characterizing mitochondrial DNA mutations in lymphocytes of people living with HIV

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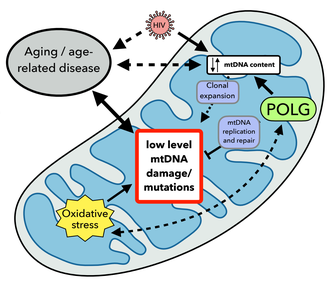
Introduction

- Both HIV and elevated mtDNA mutations are associated with aging and age related diseases
- Oxidative stress (Increased with HIV) is associated with mtDNA transversion mutations
- Mitochondrial polymerase gamma (POLG) errors are associated with mtDNA transition mutations (A↔G, C↔T)
- Lymphocytes subset composition changes with age and with immune activation due to HIV
- Very little is known about rare mtDNA mutations.
- Link between HIV, mtDNA mutations, immune aging?
- **Goal:** Detect and compare rare mtDNA mutations in lymphocyte subsets of people living with HIV and HIV-uninfected controls



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

Study Methodology

273 lymphocyte and whole-blood specimens from 49 HIV+ and 40 HIV- individuals

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DNA extraction & mtDNA barcoding

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Amplicon purification & amplification

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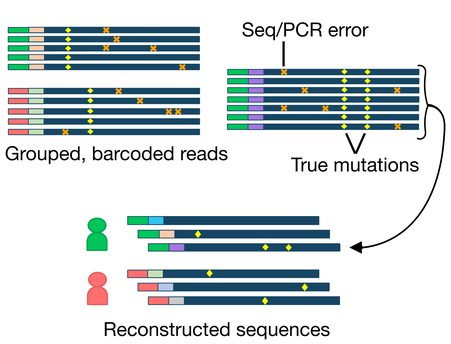
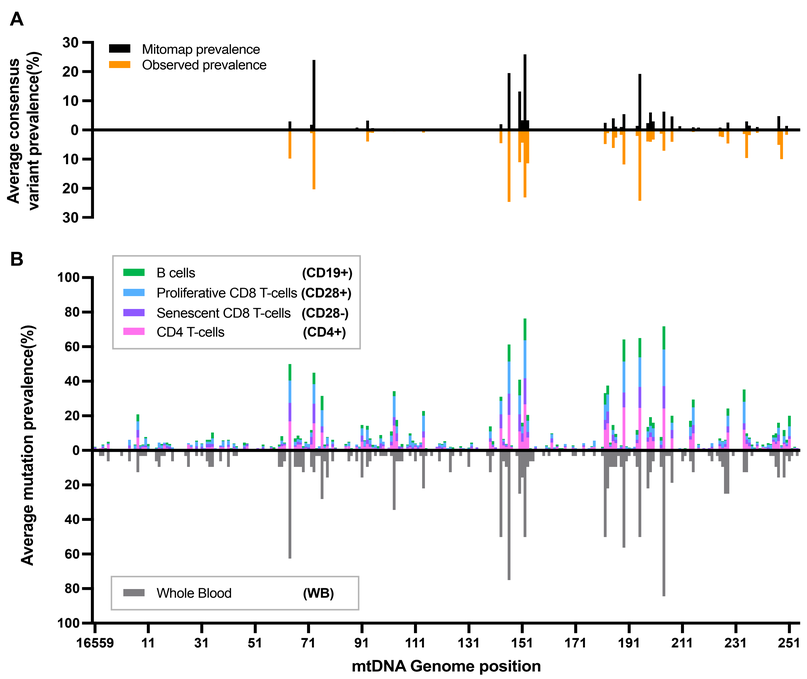
Illumina MiSeq 2x300bp Sequencing → Primer ID-based read alignment → Identification of rare mutations

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

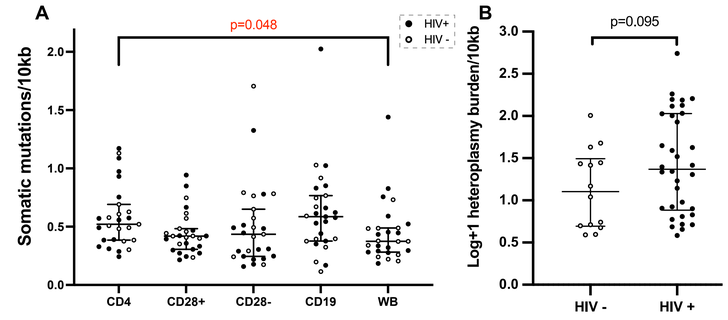


Results



▲ **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 our study specimens (orange). **B)** Average prevalence of mutations among specimens in whole blood (grey) and immune cell subsets (colours) across the interrogated region of mitochondrial genome (MT16559-MT254).

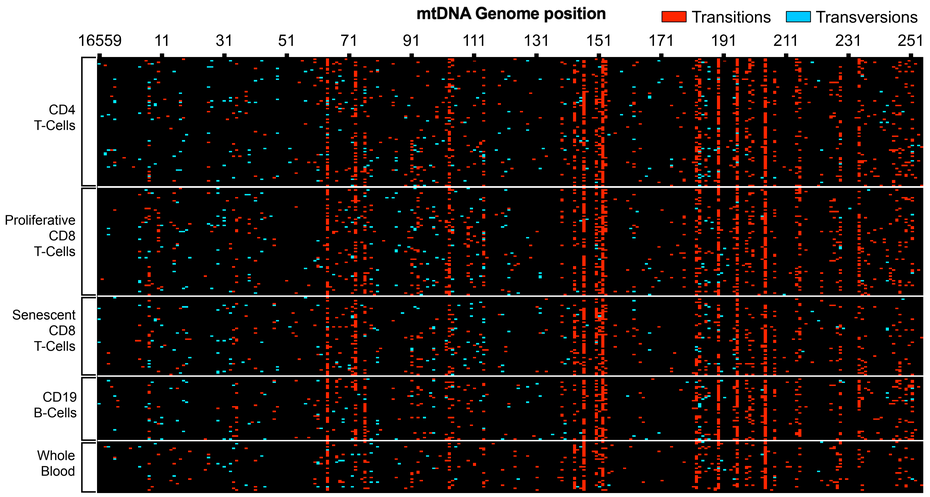
Majority of mutations are somatic (present in <1% of molecules). Hotspots (observed in ≥10% of specimens) are pronounced in whole blood and lymphocyte subsets. Conserved regions among consensus sequences of individuals appear to contain less mutations.



A) Friedman's test (p=0.022), followed by Dunn's test

B) Unpaired t-test

▲**Figure 5**: **A)** Total somatic mutation burden per 10kb among matched lymphocyte subsets **B)** Total heteroplasmy (present in ≥1% of molecules) burden per 10kb in HIV- and HIV+ individuals.



▲ **Figure 4**: Heatmap showing transition and transversion mutations for all assayed specimens.

Conclusions

- Positions tolerant to variants have a greater prevalence of rare mutations observed as hotspots, in both peripheral whole blood and lymphocyte subsets. Mutation signature is similar between lymphocytes subsets and is concordant with whole blood. **(Fig. 3)**
- Among hotspots, transition mutations are significantly more prevalent compared to transversion mutations, suggesting polymerase gamma errors may be a driver of these mutation hotspots **(Fig. 4)**
- Total mutation burden is elevated in CD4 T-cells (trend for B-cells). HIV infection does not seem to affect total somatic mtDNA mutation, but may increase heteroplasmy burden **(Fig. 5)**
- Together these findings inform future studies investigating the link between mtDNA mutations, HIV and aging

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