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THE MITOCHONDRIAL GENOME OF HEALTHY MICE AND HUMANS CONTAINS A HIGH DIVERSITY OF GENETIC VARIANTS

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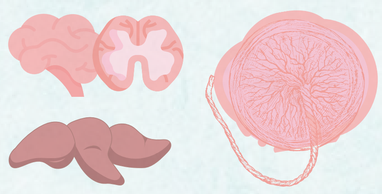
INTRODUCTION

Mutations in the human mitochondrial genome (mtDNA) cause a wide variety of debilitating and often fatal diseases. Although mutation-carrying variants in the mitochondria can coexist with normal genomes in heteroplasmy, it has been proposed that the increase in the frequency of mutations above a critical threshold (depending on the tissue) is the cause of mitochondrial dysfunction. Here, we show single nucleotide variants in mtDNA of embryonic, adult, and aged mouse brains with no overt age-related increase. We also detected *de novo* variants in oocytes and adult liver and found that in half of the human samples analyzed, over 60% of the mtDNA copies may bear lesions.

METHODS

- **Mouse tissues (brain)**
  - CD1 (adults and embryos)
  - C57BL/6J (adults and embryos)
- **Whole mouse livers (3 months old)**
- **Human placenta samples**
- **Female muse oocytes**

→ **mtDNA extraction and sequencing**



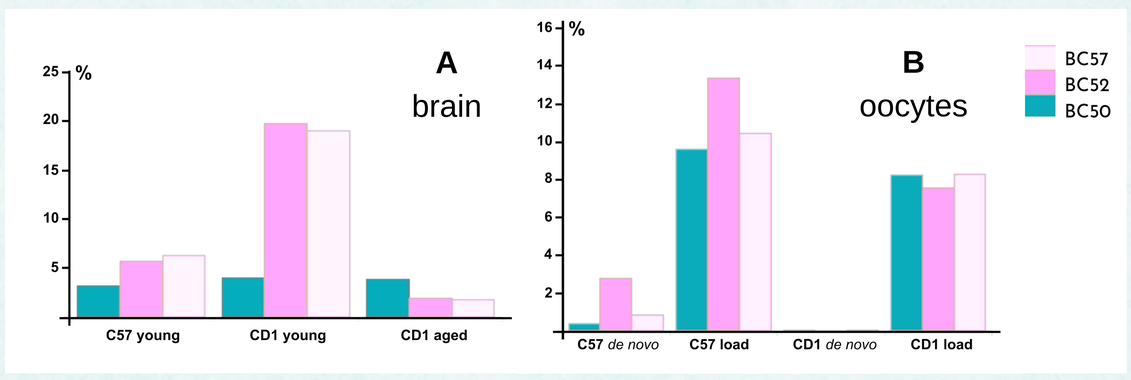
- HiSeq200 / 2500
- NextSeq550

→ **mtDNA isolation from single oocytes and sequencing**

→ **Sequence analysis**

- Trimmomatic
- Segemehl
- Mutect2
- Stringent filtering
- PhyloTree

RESULTS



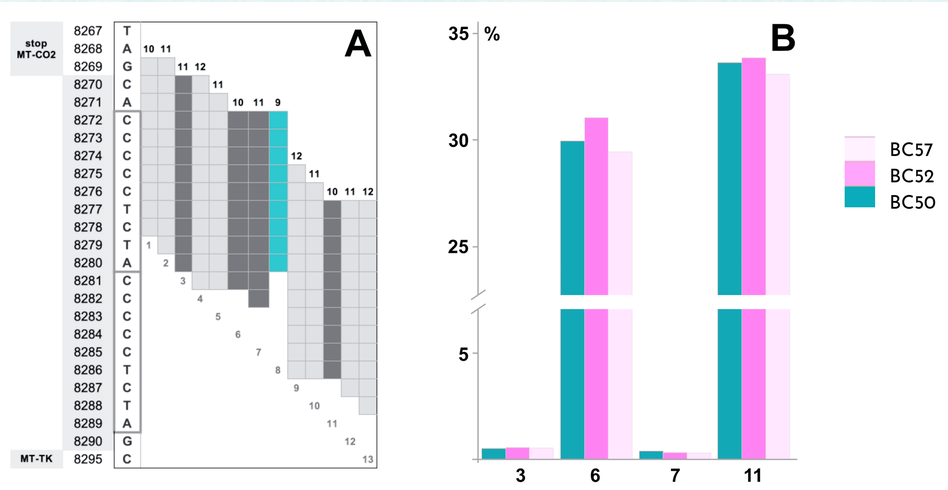
Deletion load in mouse brain and oocytes. No age-related increase in frequency for CD1 was detected in mouse brain. Total deletion load and individual variant abundance were very homogenoeus within each C57 and CD1 sample triads in mouse oocytes.

Sample	Genome Position	Major allele (MaA)	Minor allele (MiA)	MiA frequency	Refseq Codon	Variant Codon	Feature (gene, D-loop, etc.)
BC20	5369	T	C	0.024	GAT, D14	GAC, D	COX1
BC24	15338	T	C	0.016			mt-Tt
BC36	15897	T	C	0.026			D-loop
BC39	6371	T	C	0.011	TTT, F348	TTC, F	mt-Co1

Mitochondrial genome variants originated in a single generation detected in mouse liver. Samples BC20 and BC24 belong to the same pedigree. Samples BC36 and BC39 belong each to a different pedigree.

Sample	Genome position	Major allele (MaA)	Minor allele (MiA)	MiA frequency	Refseq Codon	Variant Codon	Feature (gene, D-loop, etc.)
SM1	14943	C	T	0.010	CAT, H267	TAT, Y	mt-Cytb
SM2	3455	C	T	0.015	AAC, N235	AAT, N	mt-Nd1
SM3	7464	G	A	0.010	CGT, R151	CAT, H	mt-Co2
	10993	G	A	0.010	TGC, C276	TAC, Y	mt-Nd4
SM4	16102	C	T	0.012			D-loop

Mitochondrial genome variants detected in mouse oocytes. Only samples with variants are shown. Samples SM1-SM3 are from a C57BL/6J individual and SM4 is from CD1.



Thirteen deletions with a length of 9 to 12 nucleotide were found in an intergenic region of the mitochondrial genome in human placenta. Strikingly, regardless of the abundance of deletions in this region had very similar heteroplasmy profile across BC50, BC52, and BC57.

CONCLUSIONS

The increase of age-related variants in rate could lead to their accumulation in specific cell types or structures whilst being undetectable at the organ or tissue level. As observed in this study, it appears to be that most de novo base substitutions are not related directly to oxidative damage to DNA, this strengthens the need to explore new avenues related to the theory that links the generation of variants to oxidative damage of the mtDNA followed by their accumulation as aging ensues. In half of the human samples analyzed, over 60% of the mitochondrial genome copies may bear lesions such as a group of base substitutions of low heteroplasmy in a single individual or those of the intergenic deletion cluster (IDC) which likely corresponds to the previously identified "9 nucleotide deletion".