A role for BCL2L13 and autophagy in germline purifying selection of mtDNA

Laura S. Kremer[1,*], **Lyuba V. Bozhilova[2,*]**, Diana Rubalcava-Gracia[1], Roberta Filograna[1], Mamta Upadhyay[1], Camilla Koolmeister[1], Patrick F. Chinnery[2], Nils-Göran Larsson[1]

[1] Department of Medical Biochemistry and Biophysics, Karolinska Institutet, Stockholm, Sweden

[2] MRC Mitochondrial Biology Unit and Department of Clinical Neurosciences, University of Cambridge, Cambridge, United Kingdom

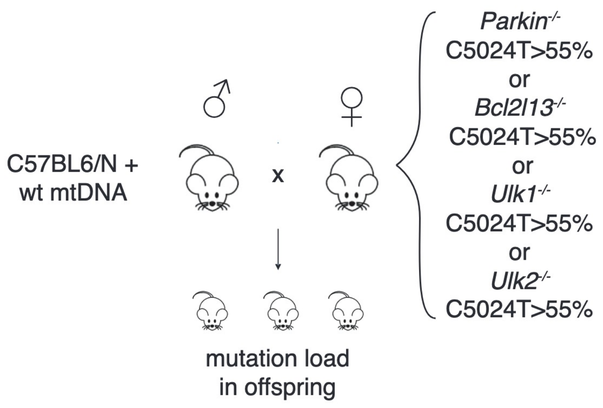
[*] Equal contribution

1 The question

- Heteroplasmy, i.e. the cooccurrence of mutant and WT mtDNA, is common in pathogenic mutations and has implications for disease phenotype.
- In mice, germline selection against the pathogenic mt.5024C>T mutation results in lower-heteroplasmy offspring.
- **Is this germline purifying selection due to autophagy, i.e. the targeted degradation of mutant mtDNA?**

2 The experiment

- We studied four genes which play different roles in mitophagy pathways.
  - ***Parkin*** is involved in ubiquitin-mediated mitophagy.
  - ***Bcl2l13*** is a mitophagy marker.
  - ***Ulk1*** and ***Ulk2*** are highly homologous autophagy-activating kinases.
- We generated female mt.5024C>T mice with homozygous knockouts of each of these four genes, as well as controls carrying the mt.5024C>T mutation only.
- **If a gene plays a role in germline purifying selection, then its knockout should result in higher-heteroplasmy offspring compared to controls.**



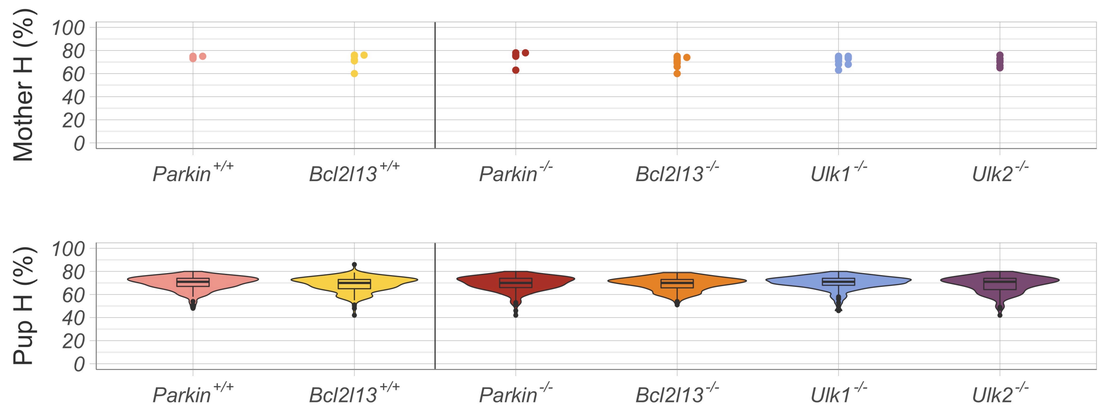
4 The answer

- We find robust evidence that *Bcl2l13*, a mitophagy marker, affects mother-to-pup heteroplasmy shift in mt.5024C>T mice.
- It is possible that a similar effect of *Ulk1* and *Ulk2* is masked by their functional redundancy.
- Overall, this suggests mitophagy plays an important role in germline purifying selection.
- **Preprint: Kremer, Laura S., et al. "A role for BCL2L13 and autophagy in germline purifying selection of mtDNA." bioRxiv (2022).**

3 The data analysis

3.1 Data overview

- A total of 959 pups born to 40 high-heteroplasmy (>55%) mothers.
- Two independent control groups, litter-matched for the *Parkin*$^{-/-}$ and *Bcl2l13*$^{-/-}$ groups.
- **As many pups (23.89 ± 10.64) are born to each mother, observations are not independent.**

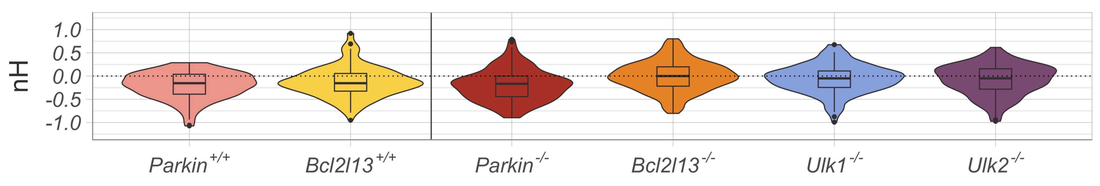


3.2 Normalised heteroplasmy shift

- Normalised heteroplasmy shift nH allows us to compare heteroplasmy changes fairly across mothers:

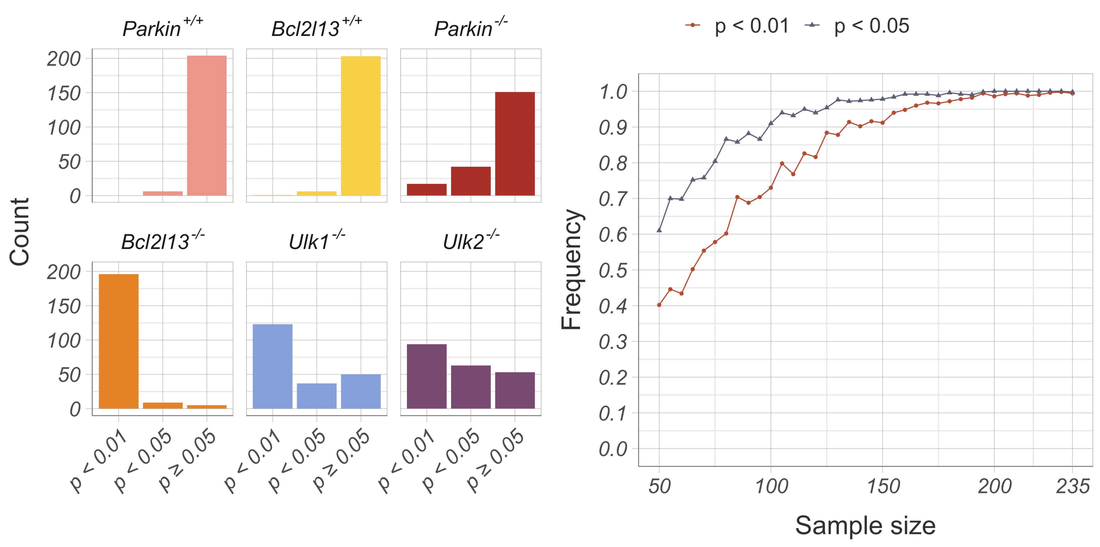
$$\mathrm{nH} = \mathrm{logit}(\mathrm{H}_{\mathrm{pup}}) - \mathrm{logit}(\mathrm{H}_{\mathrm{mother}}).$$

- Consistent negative shift $\mathrm{nH} < 0$ is evidence of purifying selection. It is observed in the control and *Parkin*$^{-/-}$ groups.
- **There is some evidence of impaired selection in the *Bcl2l13*$^{-/-}$, *Ulk1*$^{-/-}$ and *Ulk2*$^{-/-}$ groups.**



3.3 Are these results robust?

- Errors in mother heteroplasmy measurement can propagate.
- For robust results, we repeatedly compare each genotype group to a subsample of the controls (six out of ten mothers) using KS tests.
- **The *Bcl2l13* effect is observed in nearly all subsamples and under pup downsampling.** The *Ulk1* and *Ulk2* effects are seen sometimes, but not consistently.



MRC Mitochondrial Biology Unit

UNIVERSITY OF CAMBRIDGE