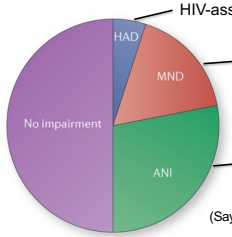
Cell-specific effects of PERK-B associated SNPs

Noah Beratan – COL 2023
Kelly Jordan-Sciutto, PhD – Department of Oral Medicine, School of Dental Medicine
Shivesh Ghura & Sarah Bond, PhD

Hypothesis

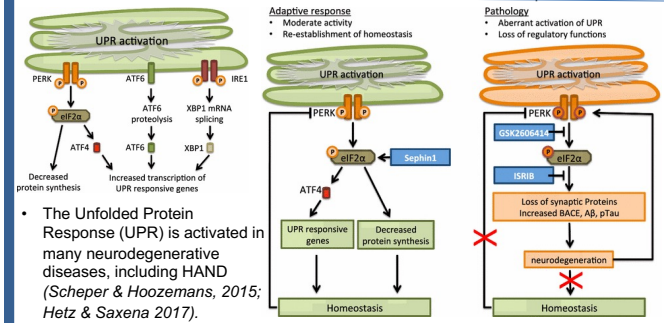
The PERK-B SNPs mediate cell type-specific changes in PERK kinase activity. In the CNS, the PERK-B coding SNPs increase PERK activation in astrocytes, negatively impacting the neurons and contributing to HAND pathology.

Background



(Saylor et al., 2016)

- Despite advances in HIV antiretroviral treatments (ART), about half of people living with HIV (PLWH) have some form of HIV-associated neurocognitive disorder (HAND) *(Saylor et al., 2016)*.
- This is linked with decreased volume of both gray and white matter in the brain and neuronal degeneration *(Irollo et al., 2021)*.
- Neuroinflammation, ER stress, and oxidative stress have been linked to this impairment.



- The Unfolded Protein Response (UPR) is activated in many neurodegenerative diseases, including HAND *(Scheper & Hoozemans, 2015; Hetz & Saxena 2017)*.
- Activation of the PERK pathway of UPR can lead to both adaptive and maladaptive responses to disease *(Scheper & Hoozemans, 2015)*.
- There is a hormetic zone where mild ER stress is protective against a second subsequent stressor *(Lu et al., 2004; Eisermann et al., 2016; Wang et al., 2017)*.

CHARTER cohort: PLWH on ART

- PERK-B carriers have higher GDS
- PERK-B has higher CSF IL6 levels
- PERK-B associated with higher beck depression index

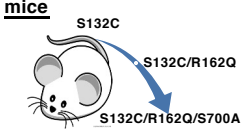
GDS	rs13045	rs867529	rs1805165
Verbal DDS	0.0809	0.0025*	0.0025*
Executive DDS	0.0131*	0.0518	0.0518
Learning DDS	0.0213*	0.1259	0.1259
Recall DDS	0.1368	0.0650	0.0650
Working memory DDS	0.5629	0.1032	0.1032
Motor DDS	0.0116*	0.0078*	0.0078*

Akay-Espinoza, 2022

- Data from the CHARTER cohort associate PERK-B with a higher global deficit score (GDS) in PLWH on ART (*Akay-Espinoza, 2022*).
- Existing data on the kinase activity of PERK-B is mixed, with some studies demonstrating increased activity and others showing reduced activity *(Liu et al., 2012; Lenh et al., 2017; Yuan et al., 2018)*.
- These contradictory results indicate that differences in PERK-B activity may be attributable to cell types and stress context.

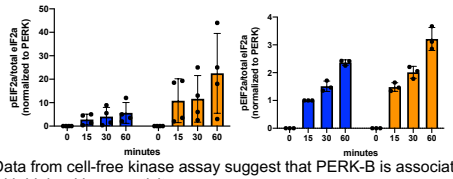
Preliminary Data

Model: Novel triple KI S132C/162Q/S700A PERK tg mice



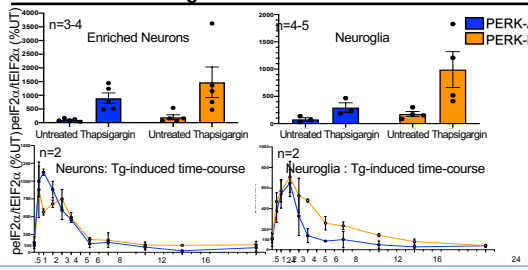
1. PERK-A/A
2. PERK-A/B
3. PERK-B/B

PERK B has higher kinase activity in a cell-free kinase assay



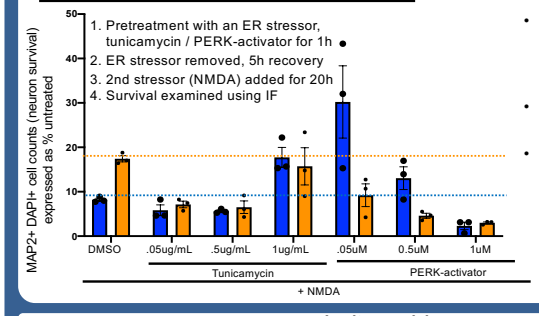
- Data from cell-free kinase assay suggest that PERK-B is associated with higher kinase activity.
- Similar trends were observed in primary mouse embryonic fibroblasts and monocyte-derived macrophages.

Neurons and neuroglia DO NOT exhibit different PERK activity



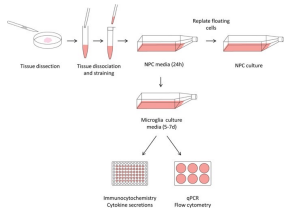
- Both PERK A/A and PERK B/B show similar levels of activation in neurons.
- In neuroglia, there is a subtle difference in PERK B/B activity, specifically wider activation kinetics. This suggests cell-specific PERK haplotype effects.
- This indicates that glia may play a critical role in PERK-mediated HAND pathology.

Altered hormesis in PERK B/B neuroglia



- Low doses of PERK-activator show hormetic-protective effects against subsequent NMDA treatments in PERK A/A neuroglia, but not in PERK B/B neuroglia.
- In contrast, a higher dose of tunicamycin exhibits hormetic effect in both haplotypes.
- While PERK B/B neuroglia show protective effects with no pre-treatment, these effects are opposite with a pre-treatment of PERK-activator, indicating the hormetic zone may have shifted in PERK B/B neuroglia, possibly due to higher background activation in PERK B/B neuroglia.

Methods



In vitro model:

- Primary neuron or neuroglial cells (DIV21-23) dissected from prenatal E18 fetuses.
- Primary mixed glia (~95% astrocytes 5%microglia) dissected from postnatal P0-3 pups.

Treatments:

PERK and UPR inducers used: **PERK Activator** (PA, mechanism unknown), **Thapsigargin** (Tg, SERCA pump inhibitor)

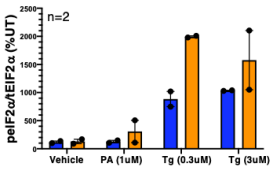
Macrophage activator used: **Lipopolysaccharide** (LPS, TLR4 activator)

Readouts:

UPR markers and cell-makers determined using western blots and immunofluorescence (IF).

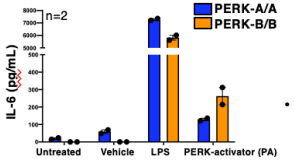
Results

PERK B/B glia show high tg-induced PERK activity



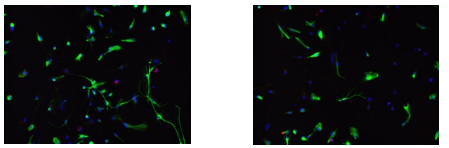
- PERK B/B mixed glia cultures are associated with increased PERK activity when treated with thapsigargin compared to PERK A/A cultures.

IL-6 secretion in PERK B/B mixed glia



- IL-6 secretion in mixed glia in the untreated, vehicle, and LPS treatments is higher in PERK A/A cells than PERK B/B cells.
- In the PERK-activator group, PERK A/A has lower IL-6 secretion than PERK B/B.

LPS-treated mixed glia 20x IF images



PERK A/A **PERK B/B**

GFAP/astrocytes (green), CD11b/microglia (red), and DAPI/nucleus (blue)

Conclusions

- When mixed glia are subject to ER stress from thapsigargin, there is a trend of higher levels of PERK activity in PERK B/B cultures compared to PERK A/A cultures.
- The inflammatory response, as measured by IL-6 secretion, to different stressors (LPS, PA) is varied, indicating that this may be context-dependent and potentially mediated by PERK-mediated hormesis.

Future Directions

- Investigate the role of PERK and PERK haplotypes in the polarization of macrophages, which serve as a model for microglia.
- Investigate how different paradigms of stress (chronic vs. acute, dose, timepoint) impact the inflammatory response in PERK A/A and PERK B/B glia, and in vivo
- Understand the mechanism underpinning PERK-B mediated changes in PERK activation and increase in IL6 secretion in vitro

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