Title: **MPL 6.8 Protein Expression in Mouse Tissues and Modified HEK293 Cells**

Background:

The mitochondrial permeability transition pore (mPTP), a transmembrane pore located in the inner mitochondrial membrane, regulates membrane potential and the proton gradient necessary to synthesize ATP. Normally closed, cellular changes such as increases in Ca2+ ion concentration, oxidative stress, or binding of regulatory proteins can open the pore, dissipating the membrane potential and inducing cell death. Cyclophilin D (CypD), a peptidyl-prolyl cis/trans isomerase mitochondrial matrix protein expressed in all mammalian tissues is a known key regulator of opening of the mPTP when bound to ATP synthase. CypD can be modified post-translationally to increase or decrease its enzymatic activity. Recent studies demonstrate that the mPTP located in the c-ring subunit is derived from ATP synthase (complex V of the electron transport chain) although mechanisms of opening the pore are still unknown. It is hypothesized that the recently discovered mitochondrial proteolipid 6.8 (MPL6.8), a hydrophobic protein with a molecular weight of 6.8 kDa, acts like a plug that seals the mPTP.

Objectives:

- Examine tissue specific differences of MPL 6.8 protein expression in adult WT and CypD -/- mice
- Examine how MPL6.8 protein expression changes through embryonic and postnatal development
- Determine how different post-translational modifications of CypD affect MPL6.8 protein expression in HEK293 cells
- Identify possible subunits of ATP synthase that MPL6.8 interacts with

Results:

MPL6.8 protein expression in isolated mitochondria from 8 tissues (heart, lung, liver, kidney, spleen, diaphragm, skeletal muscle, brain) in adult WT and CypD knockout (-/-) mice were analyzed in tris-tricine western blots (N=4). There were no statistically significant tissue specific differences detected for MPL6.8 expression when comparing WT versus CypD -/-.

MPL6.8 protein expression was then analyzed at various stages of embryonic (E) and postnatal (P) development (E11.5, E13.5, E16.5, P1, P7). Mitochondria from WT and CypD -/- mice hearts were run and analyzed in a tris-tricine western gel (N=1). There was a gradual increase of mitochondrial protein expression (VDAC, CypD) and MPL6.8 was not detected prior to P7 in the developing mouse heart.

CypD -/- human embryonic kidney cancer cell line 293 (HEK293) was transfected with mutated CypD plasmids: acetylation mimic (K166Q), deacetylation mimic (K166R), enzymatic inactive (R96G), and wildtype (WT). Tris-tricine western blots were run to analyze MPL6.8 expression in cell lysate of transfected HEK293 cells (K166Q, K166R, R96G, WTT) and untransfected HEK293 cells (WT, CypD -/-) (N=5). There was no significant difference in MPL6.8 expression between the different samples.

MPL6.8 was immunoprecipitated from samples of HEK293 cell lysate (K166Q, K166R, R96G, WTT, WT, CypD -/-) and isolated mitochondria from WT and CypD -/- adult mice heart and
kidney tissue (N=1). Results indicate that ATP5G (c-ring subunit of ATP synthase) co-precipitated with MPL6.8 in all HEK293 samples and abundantly in WT and CypD -/- mice heart mitochondria.

**Conclusion:**

CypD mutations do not affect MPL6.8 protein expression in both HEK293 cancer cells and mice tissue, more experimental trials are needed for statistical analysis. MPL6.8 expression was not detected prior to P7 in the developing heart of WT and CypD -/- mice. MPL6.8 may be associated with ATP5G (c-subunit ring of ATP synthase), supporting the hypothesis it is a plug for the mPTP in ATP synthase.