Mice lacking Cyclophilin B, but not Cyclophilin A, are significantly protected from the development of major features of NAFLD/NASH in a diet and chemical-induced model

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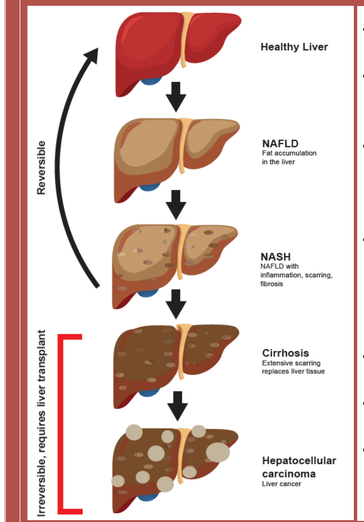
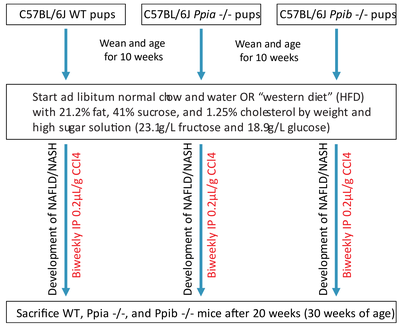
PREMISE



- ~25% of the adult population has some form of fatty liver.
- Fatty liver is associated with obesity, diabetes, and metabolic syndrome.
- Non-alcoholic fatty liver disease (NAFLD) features simple steatosis or lipid accumulation in the liver.
- NAFLD may progress to non-alcoholic steato-hepatitis (NASH) with inflammation, hepatocyte damage, and associated fibrosis.
- NAFLD and NASH can be reversed early on.
- Further scarring can induce irreversible cirrhosis and HCC
- New drug treatments are needed to prevent disease progression.

- Previous studies[1,2] have shown cyclophilin (Cyp) inhibitor drugs can prevent development of multiple features of NAFLD/NASH. It is unknown which Cyp family member's inhibition is the cause.
- Cyclophilins are a diverse family of PPIases named after their ability to bind the immunosuppressant cyclosporin A.
- **CypA** the most abundant member and is involved in many functions including immune function, cell signaling, and viral lifecycles.
- **CypB** is a protein chaperone in the endoplasmic reticulum and is associated with the unfolded protein response.

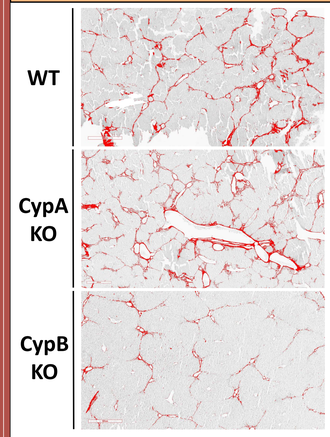
METHODS

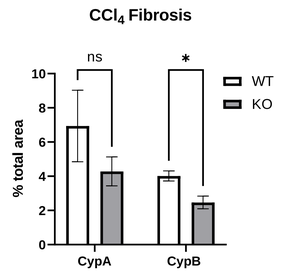


- Carbon tetrachloride (CCl_4) is an inducer of liver fibrosis.
- Mice with biweekly CCl_4 intraperitoneal (IP) with western diet (HFD), develop NAFLD/NASH.[3]
- Comparing CypA (*Ppia)* and CypB (*Ppib)* knockout (KO) mice to discrete WT control mice reveals whether CypA, CypB, or both are necessary for NASH progression.

RESULTS

CypB KO Mice Develop Less Liver Fibrosis After CCl_4 Fibrosis Model

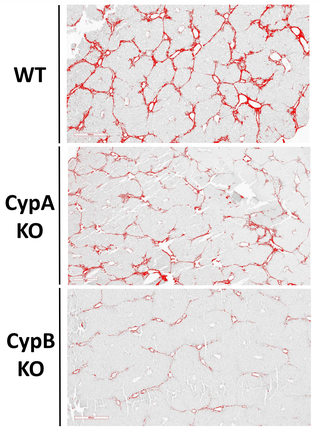


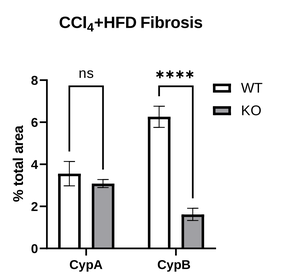


- Picrosirius red-stained liver sections from mice with biweekly CCl_4 IP injections.
- The area of stained tissue relative to the total area was measured with ImageJ software over multiple fields per sample. Representative images are shown here.

- CypA KO had significant interlobular branching fibrosis similar to WT.
- CypB KO mice however, developed significantly less.

CypB KO Mice Develop Less Liver Fibrosis After CCl_4 and HFD NAFLD/NASH Model

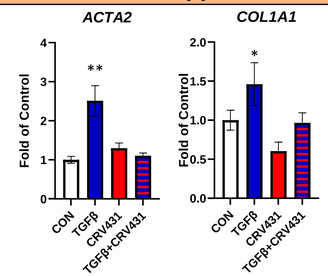




- Picrosirius red-stained liver sections from mice with biweekly CCl_4 IP injections and HFD to simulate NASH.
- The area of stained tissue relative to the total area was measured with ImageJ software over several fields per sample. Representative images are shown here.

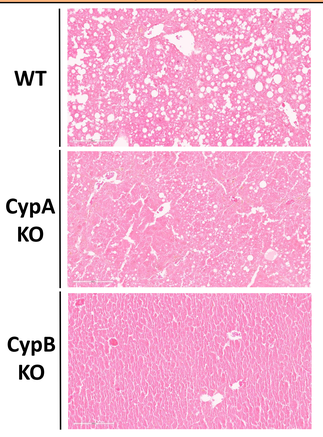
- As before, CypA KO had significant fibrosis similar to WT.
- CypB KO mice, again, displayed significantly less.

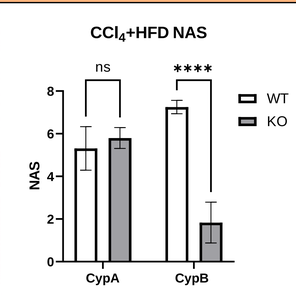
Activation Markers in LX2 Cells Stimulated by TGFβ are Suppressed with Cyp Inhibition



- Human LX2 hepatic stellate cells show increased activation markers *ACTA2* and *COL1A1* when treated with TGFβ as measured by qR-PCR. When simultaneously treated with the Cyp inhibitor CRV431, activation markers are blunted.

CypB KO Mice Have Lower NAFLD Activity Scores After CCl_4 and HFD NAFLD/NASH Model

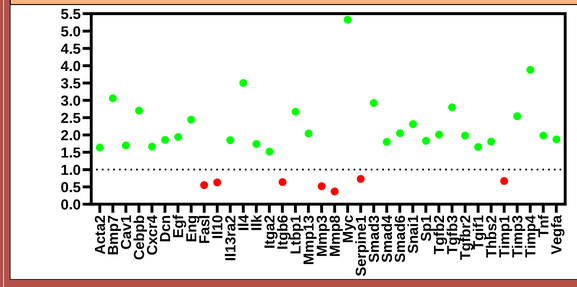




- CypA KO had a high NAFLD Activity Score (NAS), closer to WT.
- CypB KO mice however, developed few NASH features and had a low NAS.

- H&E-stained liver sections from mice with biweekly CCl_4 IP injections and HFD to simulate NASH.
- Images were scored for the three components of the NAS: steatosis, inflammation, and hepatocyte ballooning. Representative images are shown here.

CypB KO in the CCl4 and HFD Model Induces the TGFβ Antagonist *Bmp7*



- To explore possible consequences of CypB KO in NASH development, we conducted a preliminary PCR array on NASH WT and CypB KO livers of fibrosis-related gene transcripts.
- While a majority of fibrotic gene-transcripts were paradoxically upregulated in CypB KO NASH livers relative to WT, some anti-fibrosis transcripts were also increased, most especially *Bmp7*.
- *Bmp7* antagonizes the TGFβ pathway and prevents activation and proliferation of hepatic stellate cells, though it is not known whether it is regulated by CypB. Further investigation is required to confirm these findings.

CONCLUSIONS

- CypB KO, but not CypA KO, prevents the development of fibrosis in this model of CCl_4-accelerated NAFLD/NASH.
- This suggests CypB inhibition is a viable target for the treatment of fibrotic NAFLD/NASH and is in agreement with previous studies which showed global cyclophilin-inhibitor drugs had similar effects.
- Future studies include determining the mechanism(s) by which CypB KO prevents liver fibrosis and the development of NASH. Furthermore, it must be determined whether the role of CypB in the unfolded protein response or the induction of *Bmp7* play roles in this process.

REFERENCES

1. Kuo J et al. A Pan-Cyclophilin Inhibitor, CRV431, Decreases Fibrosis and Tumor Development in Chronic Liver Disease Models. *J Pharmacol Exp Ther.* 2019; 371(2):231-241.
2. Kuo J et al. Cyclophilin Inhibitor NV556 Reduces Fibrosis and Hepatocellular Carcinoma Development in Mice With Non-Alcoholic Steatohepatitis. *Front Pharmacol.* 2019; 10:1129.
3. Tsuchida et al. A simple diet- and chemical-induced murine NASH model with rapid progression of steatohepatitis, fibrosis and liver cancer. *J Hepatol.* 2018; 69(2):385-395.

DISCLOSURES

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- Stauffer and Gallay declare no competing financial interests. Ure and Foster are employees of Hepion Pharmaceuticals Inc.