Cyclophilin B knockout significantly limits the development of liver fibrosis in a diet- and chemical-induced mouse model of NAFLD/NASH

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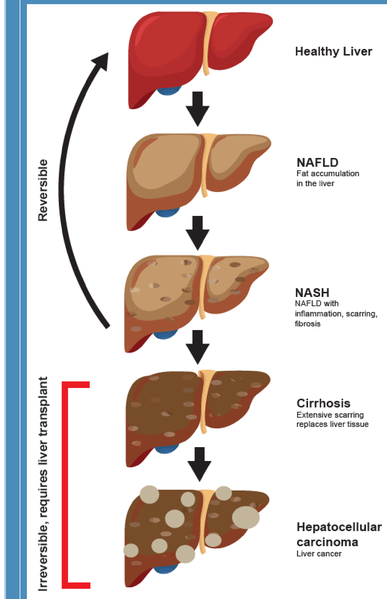
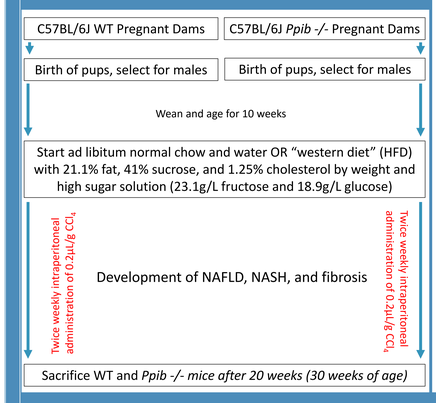
PREMISE



- ~25% of the adult population has some form of fatty liver.
- Associated with obesity, diabetes, and metabolic syndrome.
- Non-alcoholic fatty liver disease (NAFLD) features simple steatosis or lipid accumulation.
- NAFLD may progress to non-alcoholic steato-hepatitis (NASH) with inflammation, hepatocyte damage, and associated fibrosis.
- NAFLD and NASH with limited fibrosis can be reversed.
- Further scarring can induce cirrhosis and HCC which cannot be reversed.

- Previous studies[1,2] have shown cyclophilin (Cyp) inhibitor drugs can prevent development of fibrosis. It is unknown which Cyp family member(s) is the cause.
- Cyclophilins are a family of peptidylprolyl isomerases with diverse functions. CypB is a protein chaperone in the endoplasmic reticulum and is associated with the unfolded protein response.

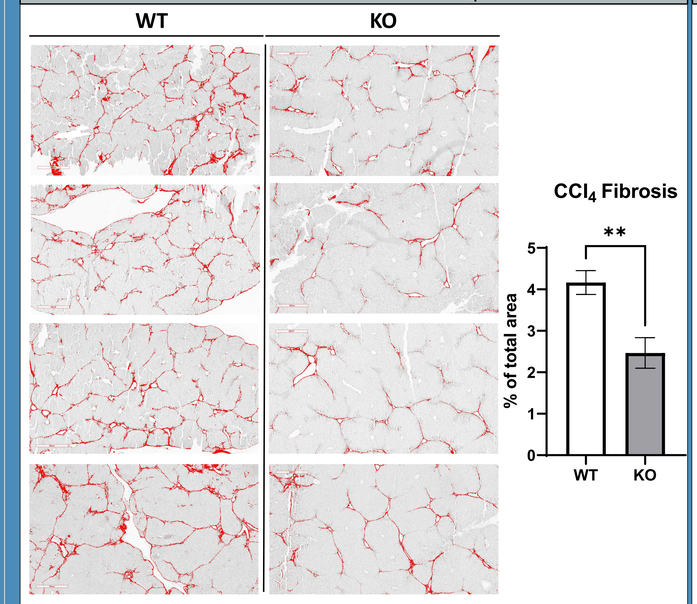
METHODS



- Carbon tetrachloride (CCl_4) is a potent inducer of liver fibrosis
- CCl_4 with western diet (HFD), reliably produces many features of NAFLD/NASH in mice.[3]
- Comparing wild-type (WT) mice to *Ppib* (CypB) knockout (KO) mice reveals a necessity for CypB in liver fibrosis.

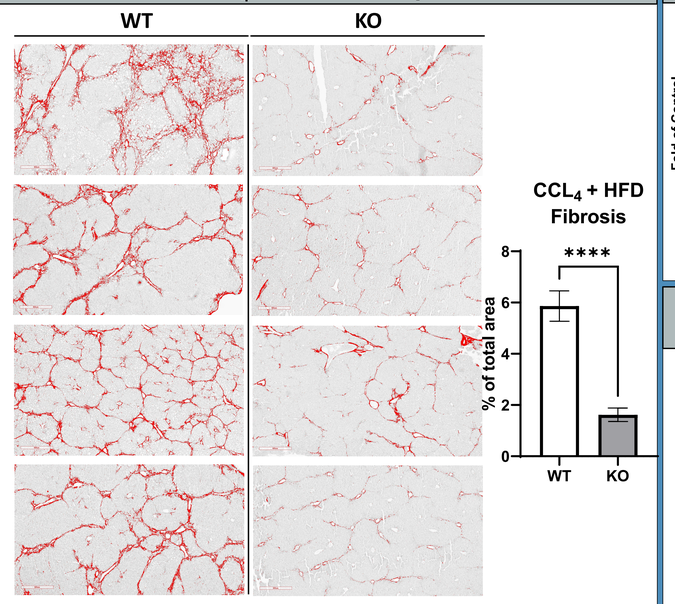
RESULTS

CypB KO Mice Develop Less Liver Fibrosis After Administration of CCl_4



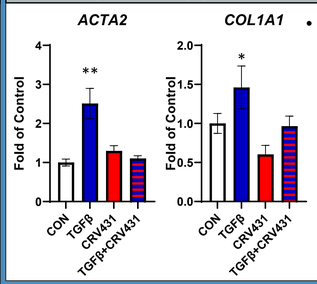
- With CCl_4 twice weekly intraperitoneal (IP) administration alone, both WT and CypB KO mice developed significant interlobular branching fibrosis, as determined by histological analysis of pirco-Sirius red-stained liver tissue sections.
- KO mice however, displayed significantly less stained area relative to WT.
- 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.

CypB KO Mice Develop Less Liver Fibrosis After Administration of CCl_4 and HFD to Replicate NAFLD/NASH



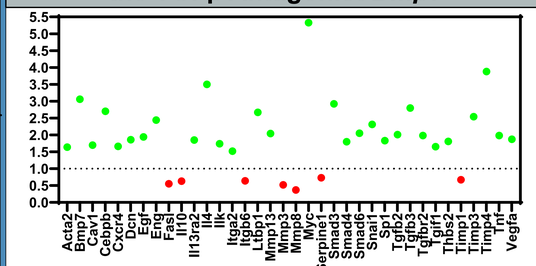
- When CCl_4 twice weekly intraperitoneal (IP) administration was combined with "western" or high-fat diet (HFD), WT mice developed significantly more interlobular branching fibrosis, as determined by histological analysis of pirco-Sirius red-stained liver tissue sections.
- KO mice however, displayed significantly less stained area relative to WT, to an even greater degree than with CCl_4 only.
- 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.

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 in the CCl4/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 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 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) of action of CypB KO in preventing fibrotic deposition. 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, Kuo, Bobardt, and Gallay declare no competing financial interests. Ure and Foster are employees of Hepion Pharmaceuticals Inc.