Differential expression of cardiac fibrosis genes in response to chronic and binge alcohol consumption in male C57BL6/J mice

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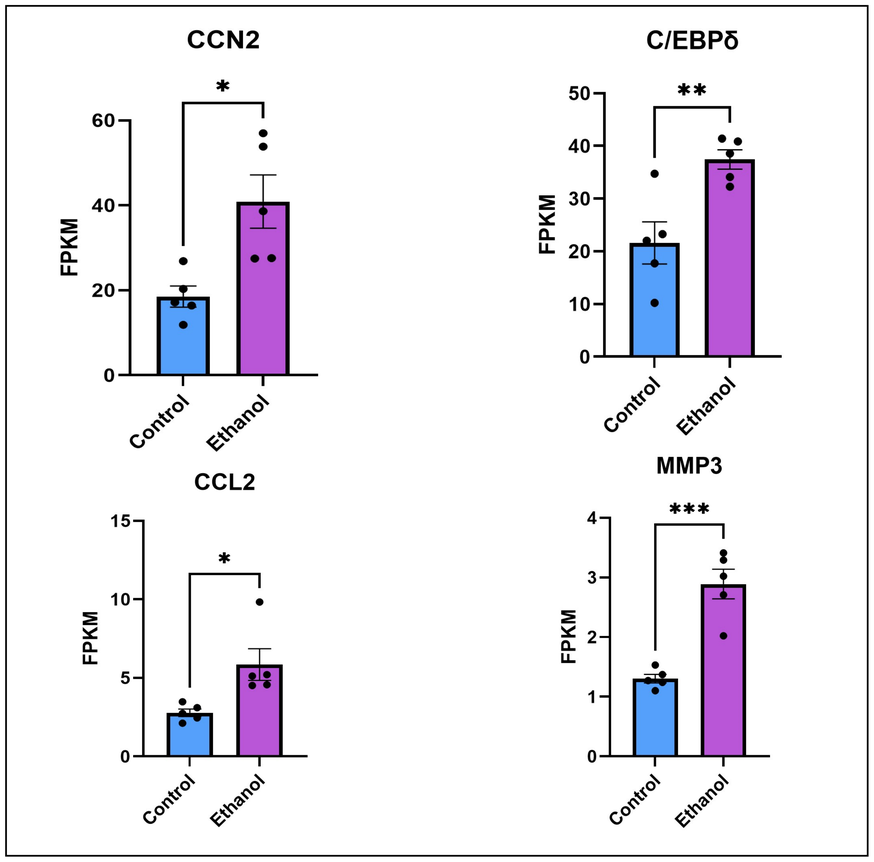
Introduction

- Binge drinking can cause permanent injury to many tissues in the body, such as the liver and the gastrointestinal, pulmonary, and cardiovascular systems, and the cardiac effects of chronic alcohol consumption on the heart can be severe.
- Alcohol-induced cardiomyopathy (ACM) is a leading cause of non-ischemic dilated cardiomyopathy, which is characterized by both systolic and diastolic failure. Prior to systolic dysfunction, chronic alcohol use causes fibrosis and diastolic dysfunction in the heart. However, there are few studies on the regulation of the fibrotic process in ACM.
- Objective: To study the differential pro-fibrotic gene expression in a preclinical model of ACM.
- Hypothesis: Mice exposed to chronic and binge alcohol feeding will have increased expression of profibrotic genes.

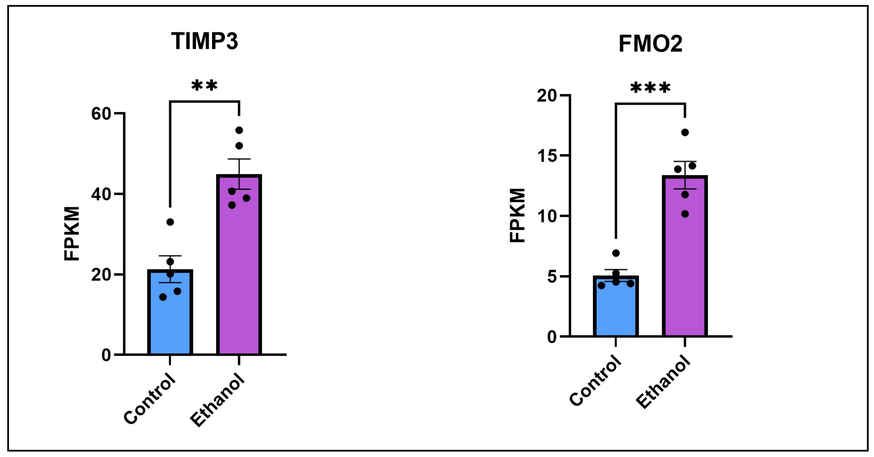
Methods

- Male C57BL6/J mice were fed the Lieber-Decarli liquid diet for 30 days with or without 5% ethanol
- In addition to the chronic 30-day feeding, the mice were given ethanol binges by oral gavage on days 10 and 30. The ethanol-exposed mice were given a binge ethanol dose of 5 g/kg, while the control mice were given a gavage of isocaloric maltose dextrin.
- On day 32, 48 hours after the second gavage feeding, the mice were sacrificed, and hearts were collected. RNA was isolated from the left ventricle and sent for sequencing. RNA expression was then used to study the regulation of cardiac fibrosis in our preclinical model of ACM.

Differential expression of pro-fibrotic genes



Differential expression of anti-fibrotic genes



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Role of altered genes in fibrosis

- Cellular Communication network factor 2 (CCN2) – plays an important role in cell growth, inflammation and ECM remodeling. Expression of CCN2 is enhanced by TGF-β, and the two proteins work together to promote fibrosis
- CCAAT/enhancer binding protein δ – a transcription factor that can upregulate expression of CCN2. Higher levels of CCN2 can enhance fibrotic processes through the TGF-β pathway.
- Chemokine ligand 2 (CCL2) is a proinflammatory chemokine known to play a role in fibrosis. CCL2 attracts monocytes with the CCR2 receptor, which release profibrotic mediators that can activate cardiac fibroblasts.
- Matrix metalloproteinases are enzymes that break down collagen fibers and other ECM components. The breakdown of ECM proteins allows for matrix remodeling, and elevated MMP levels are often associated with cardiac fibrosis.
- Tissue inhibitor of metalloproteinase 3 is a regulatory protein that reduces activity of multiple MMPs. Low levels of TIMP3 have been associated with extensive fibrosis in mouse models, indicating that TIMP3 has an anti-fibrotic effect.
- Flavin-containing monooxygenase 2 has been shown to decrease activity in the TGF-B pathway by inhibiting phosphorylation of SMAD2 and 3. Rodent models have shown that loss of FMO2 is a significant contributor to cardiac fibrosis.

Conclusion

- Analyzing the relative expression of genes in the ethanol-exposed mice hearts revealed multiple profibrotic genes significantly upregulated.
- CCN2, CCL2, C/EBPδ and MMP3 are all proteins associated with fibrotic processes in the heart and were found at elevated levels in the alcohol group.
- There were also genes with anti-fibrotic effects that had altered expression, notably FMO2 and TIMP3, which were significantly elevated.
- These findings reveal a balance of pro-fibrotic and anti-fibrotic effects taking place in heart tissue exposed to chronic alcohol