AKR1D1 knockout mice show signs of dysbiosis, intestinal damage and increased gut permeability

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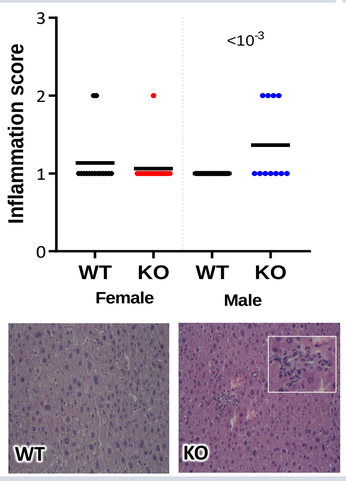
Introduction

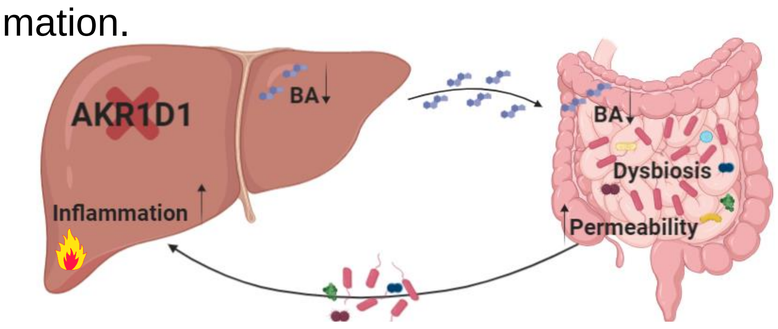
Gut dysbiosis and metabolic endotoxemia contributes to the progression of non-alcoholic fatty liver disease (NAFLD) from simple steatosis to steatohepatitis. Bile acids (BA) are potent antimicrobials that support gastrointestinal health, and the dysregulation of BA homeostasis in NAFLD is hypothesised to drive dysbiosis.

5β reductase (AKR1D1) is a key enzyme in the BA synthesis pathway. We have previously shown that AKR1D1 expression and activity is decreased in NAFLD and have generated a novel AKR1D1 knockout (KO) mouse. Histology at 52-weeks revealed a trend towards increased hepatic inflammation in the female AKR1D1 KO mice.



Hypothesis

We predict that AKR1D1 deletion will reduce hepatic and intestinal bile acids levels leading to dysbiosis and intestinal damage and resulting in increased intestinal permeability and metabolic endotoxemia, driving hepatic inflammation.



Methods

Female and male wildtype (WT) and AKR1D1 KO mice were maintained on a control diet until 52-weeks of age. Liver BA level and composition were determined by LC-MS/MS and faecal BA levels by ELISA. Cecal microbiome composition was determined by 16s rRNA analysis. Histological characterisation was performed on H&E stained small intestine (ileum). At a molecular level, expression of key tight junction genes (*Claudin 1, Occludin, Zonula occludens 1*) in the intestine (ileum), bacterial DNA, pattern recognition receptor *Tlr4,* myeloid differentiation primary response 88 *(Myd88),* tumor necrosis factor alpha *(Tnfa)* and nuclear factor NF-kappa-B *(Nfkb1)* in the liver, were measured by qPCR and western blotting. All data were analysed by student's t test (n=7-16).

Results

AKR1D1 KO mice have decreased liver and faecal BA levels

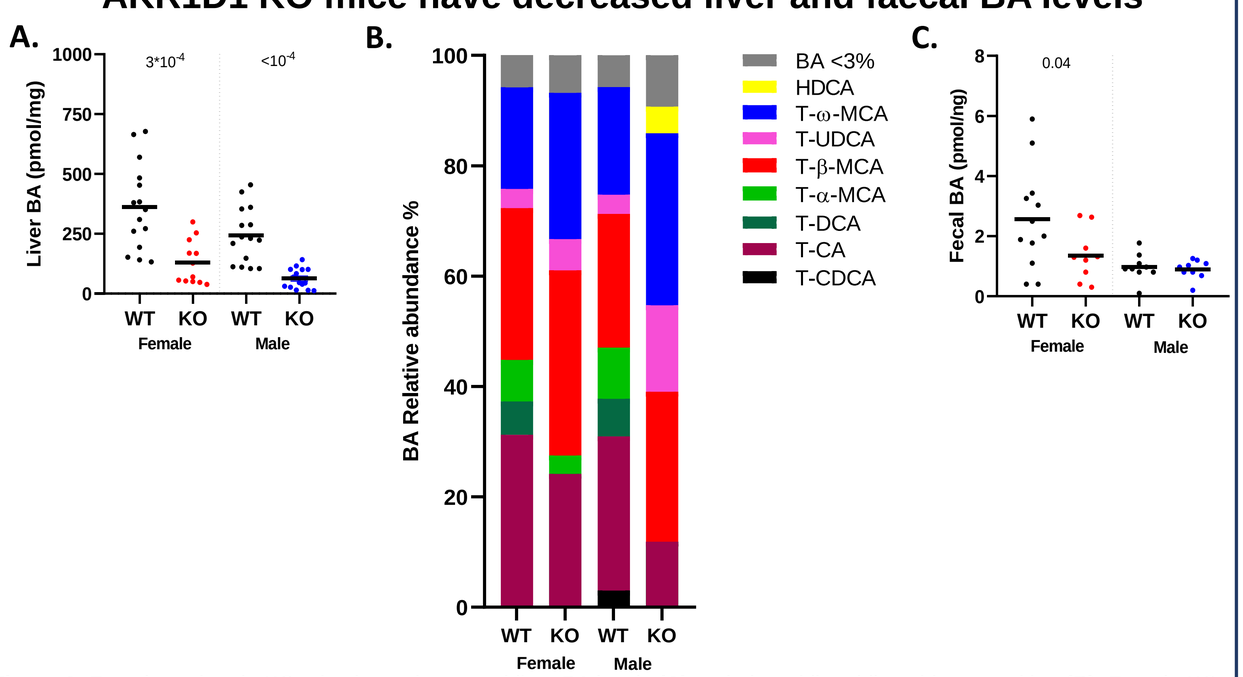


Figure 1. Female and male KO mice have decreased liver BA levels **(A)** and altered liver bile acid composition **(B)**. Female KO, but not male, mice have decreased faecal BA levels **(C).**

AKR1D1 KO mice have altered microbiome composition

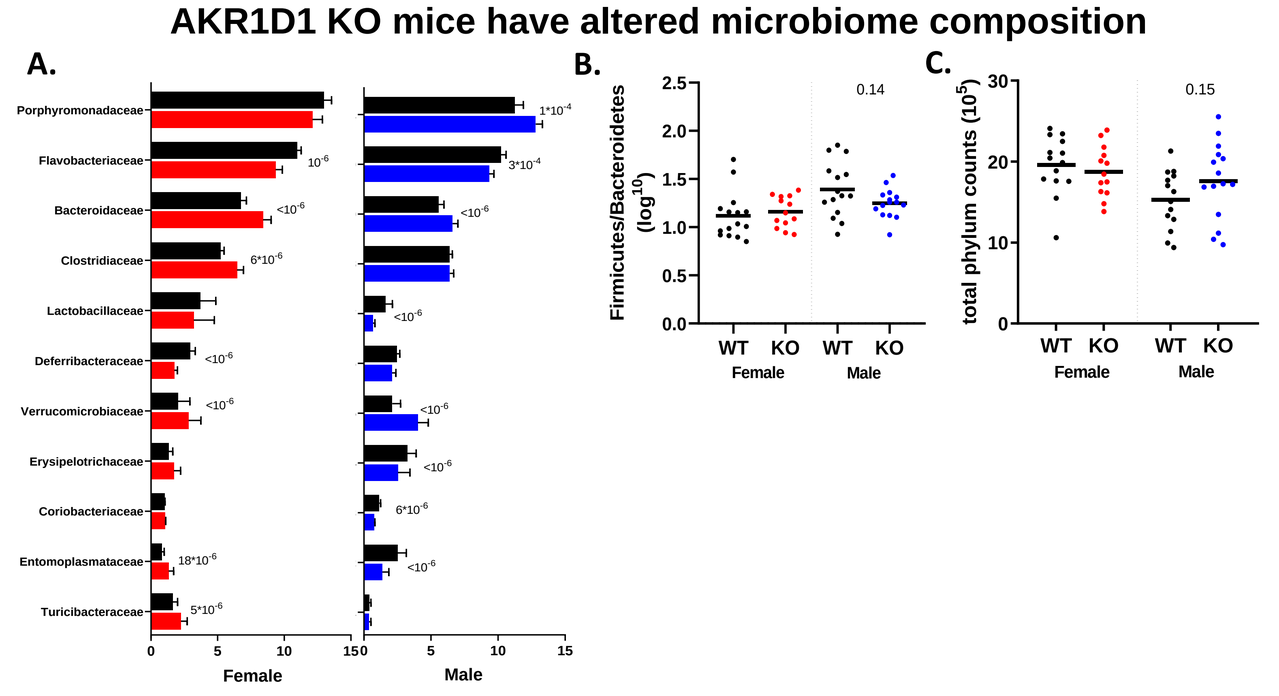


Figure 2. Female and male KO mice have family level changes in microbial composition **(A)** with a trend towards decrease in Firmicutes / Bacteroidetes ration **(B)** and increase in Total phylum counts in male KO animals **(C)**.

AKR1D1 KO mice show evidence of intestinal damage but no sign of inflammation or apoptosis

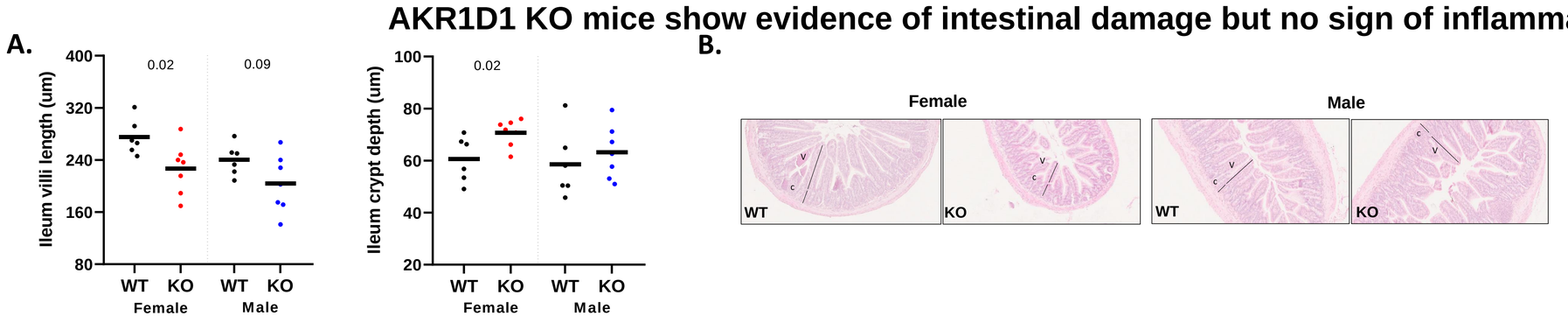
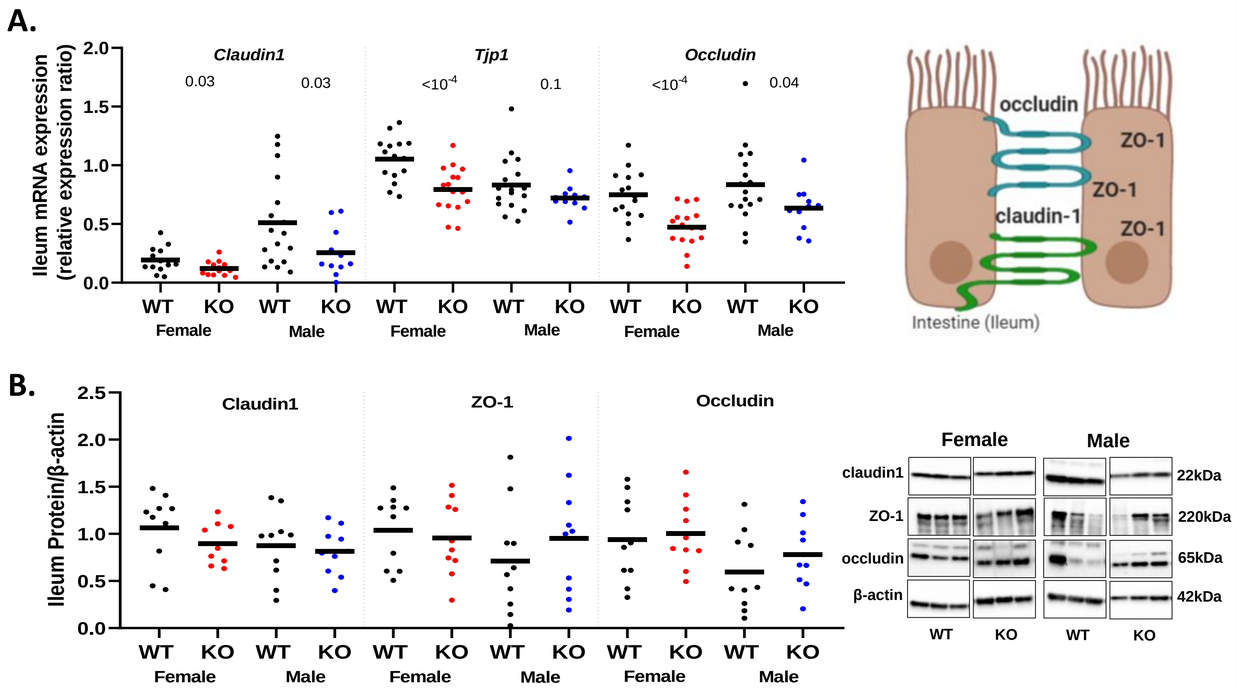


Figure 3. Female KO mice have shorter ileal villi and longer crypts with male mice following the same trend **(A).** Representative pictures of ileum sections, villi (V), crypt (c) **(B)** .Female and male KO mice have unchanged expression levels of *Tnfa* and *Casp3* **(C)**.

Gene expression but not protein level of tight junction proteins is decreased in AKR1D1 KO mice



AKR1D1 KO mice have increased amount of bacterial DNA in liver followed by increased TLR4, *Nfkb1* and *Tnfa* expression in females

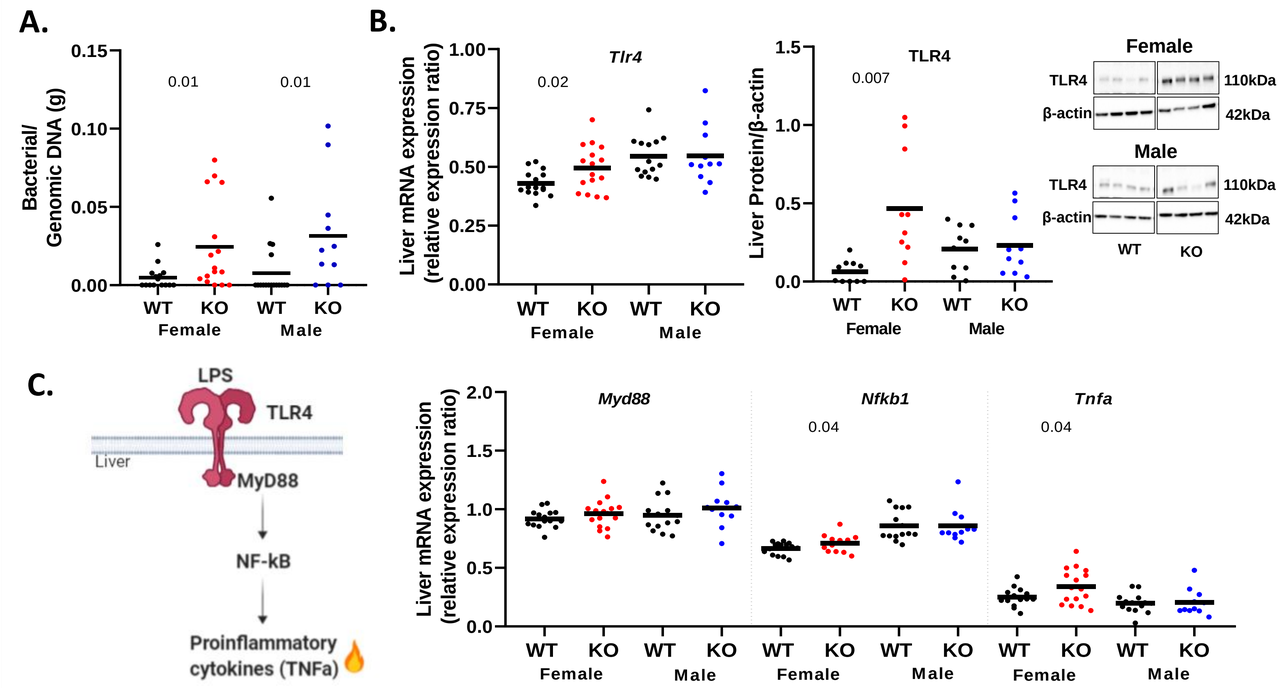


Figure 4. Female and male KO mice have decreased *Claudin 1*, *Occludiin* gene expression and female KO have decreased *Tjp1* levels **(A)**. Protein levels are comparable to WT animals **(B)**.

Figure 5. Female and male KO mice have increased amount of bacterial DNA in the liver compare to WT **(A)**. Female but not male KO mice have increased hepatic TLR4 gene and protein levels **(B)** along with increased gene expression of *Nfkb1* and *Tnfa* **(C)**.

Conclusion

AKR1D1 deletion results in decreased liver BA levels and altered composition. Only female KO mice had reduced faecal BAs, but both sexes showed altered microbiome composition. Changes in microbiome was accompanied by signs of intestinal damage with shorter ileal villi and longer crypts, reaching significance only in female KO animals. Intestinal gene expression markers of inflammation and apoptosis were unchanged. Suggestive of increased intestinal permeability, expression of tight junction genes *Claudin 1*, *ZO-1* and *Occludin* were reduced, although this did not translate to protein. Furthermore, in the liver, KO animals showed signs of endotoxemia with increased presence of bacterial DNA compare to WT. Interestingly the TLR4 pathway of innate immunity was activated only in the female KO animals with increased mRNA and protein expression of TLR4 and TLR4 mediated genes, *Nfkb1* and *Tnfa*. Collectively, our data suggest that reduction in AKR1D1 activity, as seen in NAFLD patients, may contributes to dysbiosis and metabolic endotoxemia.

References

[1] Shapiro H.,, et al,.Bile acids in glucose metabolism in health and disease. The Journal of Experimental Medicine (2018). [2] Peng J., et al,.Geniposide and Chlorogenic Acid Combination Ameliorates Non-alcoholic Steatohepatitis Involving the Protection on the Gut Barrier Function in Mouse Induced by High-Fat Diet. Frontiers in Pharmacology (2018) [3] Kemis, J. H., et al,.Genetic determinants of gut microbiota composition and bile acid profiles in mice. PLOS Genetics (2019)