# High throughput RNA Seq Unravels Pathways Associated with Cognitive Deficit in Primary Billiary Cholangitis

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## Background

- Primary Billiary Cholangitis (PBC) is an autoimmune disease of the bile duct and liver which causes biliary epithelial cell injury.
- Leading to cholestasis, fibrosis and circulation of toxic bile acids. >1/3 patients report severe, life limiting cognitive deficits.
- Previous work from the lab shows bile duct ligated (BDL) mice which develop cholestatic liver disease also exhibit cognitive deficits in hippocampal-dependent behavioural tasks such a novel arm Y-maze.
- In this model, circulatory bile acids cause disruption to the Blood-Brain Barrier (BBB) **(Fig. 1)** leading to cognitive symptoms **(Fig. 2)**. The pathways associated with these deficits within the brain have been poorly characterised.

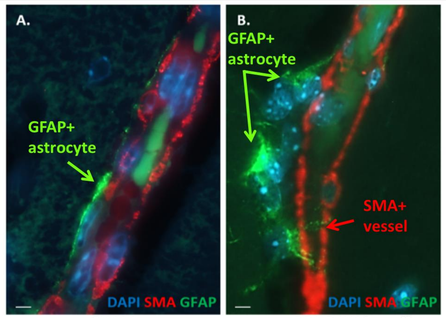


**Figure 1.** Super resolution imaging from the mouse BDL study showing disruption to the BBB forming astrocytes

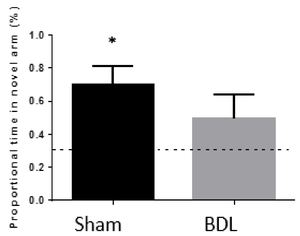


**Figure 2.** Y-maze testing in sham versus BDL mice, shows a decline in visuo-spatial memory with BDL ( p<0.05)*

## Aims

**To investigate the mechanistic basis of cholestasis-induced memory impairment in mice and discern efficacy of the FDA-approved drug Obeticholic Acid (OCA) in ameliorating symptoms, using RNA sequencing.**

## Methods

- C57BL/6 mice underwent either Bile Duct Ligation (BDL) or sham surgery.
- BDL sub-groups were treated prophylactically (-3 days) or therapeutically ( +3 days) with OCA **(Fig 3)**.
- Animals were humanely killed at day 10 and brains removed for hippocampal biopsy.

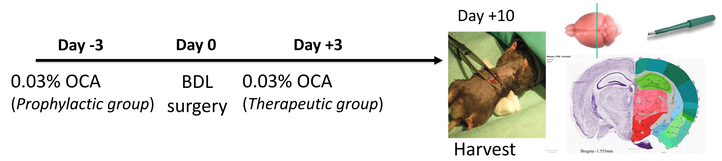


**Figure 3**. BDL surgery and dosing timeline

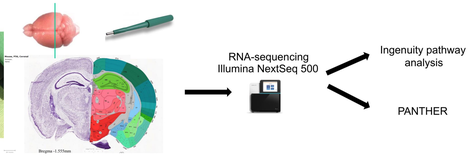


**Figure 4**. biopsy sequencing and analysis workflow

Punch Biopsy was taken from dorsal hippocampus sectioned coronally at -1.555mm Bregma. RNA was isolated from each biopsy sample and processed for RNA sequencing using an Illumina NextSeq 500 system. Differential expression analysis was carried out in R, with the packages DESeq2 v1.20.0[3]. Multiple test correction was done with IHW (version 1.8.0). Genes were judged significantly DE that have adjusted p. value <= 0.05 and |log2(fold-change)| > 1**.** Pathway analysis was performed using PANTand Ingenuity pathway analysis (IPA) software **(Fig. 4)**.

## Conclusions

1. **Cholestasis correlates with reduced neuronal plasticity and long-term potentiation (suggesting memory problems) in this model.**
2. **OCA treated animals have a deficit reversal and hippocampal transcriptome similar to Sham operated animals.**
3. **These data suggest that OCA treatment may be able to help reverse the effects of cholestasis in the brain and promote neuronal regeneration in the hippocampus, a region that controls memory.**

**References: 1.** Newton, J. L. *et al* (2008), Cognitive impairment in primary biliary cirrhosis: Symptom impact and potential etiology. *Hepatology.* 48: 541-549; **2.** Young SN, Shalchi M (2005). The effect of methionine and S-adenosylmethionine on S-adenosylmethionine levels in the rat brain. *J Psychiatry Neurosci*. 30(1):44-8; **3.** Love, M. I. (2014) Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biol.* 15(12):550.

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## Results

- Both prophylactic and therapeutic administration of OCA significantly improved cognitive function of the mice vs control as tested using Y-maze. This test relies on visuo-spatial memory and uses time spent in novel arm as the primary measure) **(Fig. 5).**

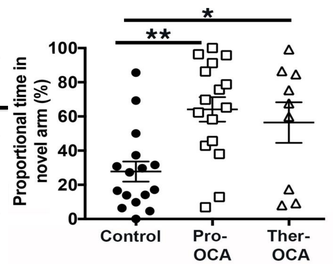


**Figure 5.** OCA treatment improved visuo-spatial memory at day 9 in mice compared to BDL control groups (p<0.01) **( p<0.05)*

- Differentially expressed (DE) gene lists were generated by comparing the hippocampal transcriptome of sham mice with BDL +/- OCA treated mice. In the BDL control group, 1376 genes were DE compared to sham animals, whereas 551 and 774 genes were DE in mice given either prophylactic or therapeutic OCA **(Fig. 6).** 869 genes were unique to the BDL control group, whilst 443 were OCA-dependant. Only 220 DE genes were overlapping between the three groups.
- Panther molecular function analysis of DE gene lists from BDL control or BDL ProOCA treated mice compared to sham mice revealed that signalling pathways were equally activated in both groups highlighting the effect of the therapy in maintaining relatively normal cognitive functions **(Fig 7).**

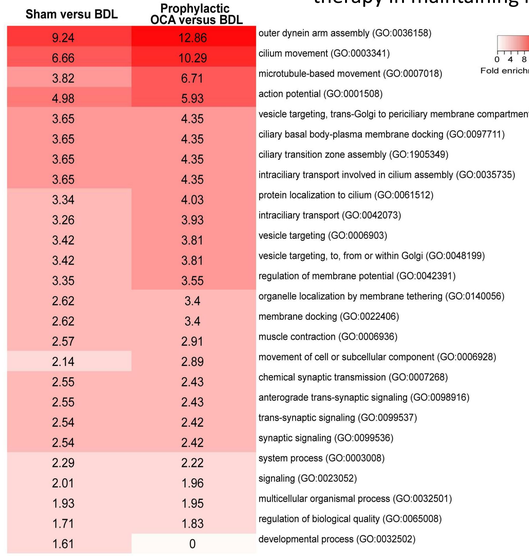


**Figure 7**. Heat map of activated pathways (Panther analysis) in control and proOCA treated groups.

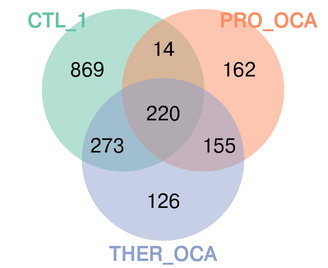


**Figure 6**. Venn diagram of the overlapping versus unique DE genes compared to sham mice, between the BDL control, proOCA and therapeutic OCA treated groups.

- Ingenuity pathway analysis (IPA) of DE list from the control group identified deactivation of key cognitive related pathways including apoptotic pathways, synaptic long term potentiation and cAMP signalling associated neuronal plasticity pathways such as dynein and microtubule transport and S-adenosyl methionine biosynthesis pathways[2].
- Transcriptomics of the OCA-treated BDL animals showed a reversal of these pathways and an upregulation of neuronal differentiation pathways and outgrowth genes including GATA3 and Gli1 **(Fig. 8).** Canonical WNT signalling was also increased, suggesting a reparation of long-term potentiation pathways.


**Figure 8**. IPA Pathway component analysis showing upregulated genes in the differentiation of neuron pathway.

- Immuno-fluorescent staining of the hippocampus showed a significantly higher protein expression of SOX2 (a marker of neuronal differentiation)in OCA treated BDL mice when compared to BDL animals **(Fig. 9).**

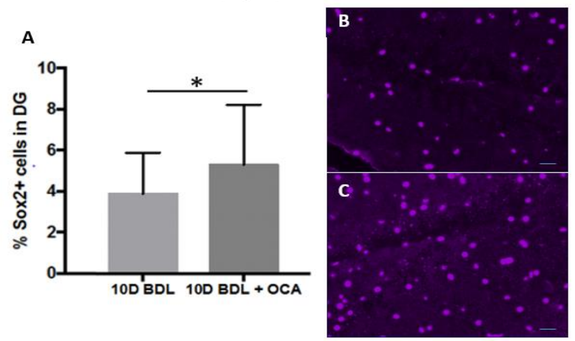


**Figure 9.** Immunofluoresent staining of SOX2 in the hippocampus showed an increase of neural progenitor cells in prophylactically OCA treated animals versus BDL ( p<0.05)*

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Cholestasis and autoimmune
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