Transcriptional profiling shows stronger regulation of peripheral immune cell subsets in patients with well-controlled primary biliary cholangitis

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Background: Primary biliary cholangitis (PBC) is an autoimmune disease characterised by progressive biliary damage leading to cholestasis, fibrosis and eventually cirrhosis.

First line therapy for PBC is UDCA, however ~ 30% of patients do not respond (non-responders); showing inadequate response to UDCA after 1 year

Our group has previously confirmed that UDCA response strongly predicts outcome in PBC; that PBC patients exhibit distinct treatment response trajectories that may reflect discrete underlying disease mechanisms, and that future UDCA response may be predicted at baseline.

GWAS studies have also identified dozens of risk loci with genes highly expressed in immune cells, suggesting that dysregulation of gene expression in immune cells is involved in PBC.

Aim: As part of UK-PBC, we sought to characterise the immunobiology of the UDCA response by correlating gene expression in peripheral immune cells with biochemical response to therapy.

Method: We undertook transcriptional profiling using RNA sequencing of peripheral blood mononuclear cells (PBMCs); monocytes, Th1 cells, Th17 cells, Tregs and B cells from 31 patients stratified by their biochemical response to UDCA, 16 with highly active disease on treatment ('non-responders', (NR)) versus 15 with minimally active disease on treatment ('responders', (R)). 15 sex matched controls (CN) were also selected for comparison.

RNA sequencing alignment and statistical analysis was completed with STAR and DESeq2 packages

Weighted Gene Co-expression Network Analysis was used to identify gene co-expression networks (so-called 'modules') associated with response status, and the most strongly associated genes (so-called 'hub genes') within them.

Results: We identified modules associated with response or non-response across all cells subsets (adjusted P-value <0.05). Associated modules and their hub genes suggest that in responders, there is suppression of monocytes (e.g. downregulation of *CEBPD*); stronger regulation of T_H1 cells (e.g. downregulation of co-stimulatory molecules, including *FLT3*, *SYK* and *TNFRSF21*); and greater activity counterbalanced by stronger regulation of T_{REG} cells (e.g. upregulation of *CTLA4*; and downregulation of *SEMA7A*, *EPHA2*, and *FCER1A*).

Results: CD14+ monocytes 19 modules were identified. The salmon module correlated with NR vs CN showed upregulation of genes and hub genes (most strongly associated genes) involved in activation of NF-KB, genes involved in differentiation and mitogenesis and key mediators of inflammation. Turquoise module showed negative correlation and downregulaton of genes in R vs CN in genes involved in inflammation and genes required for programmed necrosis. Suggesting monocytes are active and pro-inflammatory in UDCA resistant PBC also that monocyte activity might be suppressed in UDCA- responsive PBC.

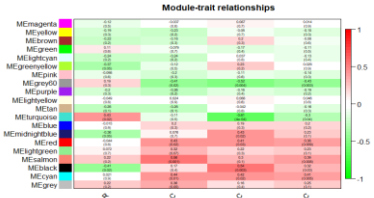
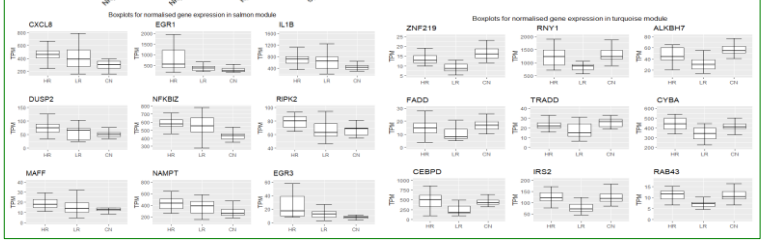


Figure 1. CD14+ cells. Module trait relationship table. Each row corresponds to a module and each column to a trait of interest. Values in the cells illustrate Pearson's correlation coefficient (PCC) and p value.
Figure 2. Boxplot of normalised gene expression for selected genes in NR, R, and CN in the salmon and turquoise module



Treg cells 9 modules were identified; pink, green and brown modules were associated with one or more traits. Pink and Green modules were upregulated in R vs CN and showed genes involved in activation of NF-KB and also in Treg cell trafficking and suppressive function. Brown module was negatively correlated with R and CN and NR vs CN and included genes such as IRF8 and FLT3. Suggesting Treg cells are activated yet restrained in PBC.

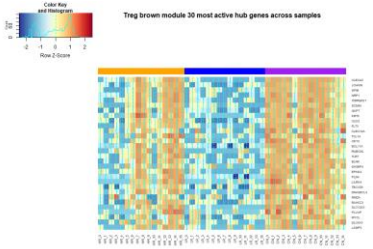
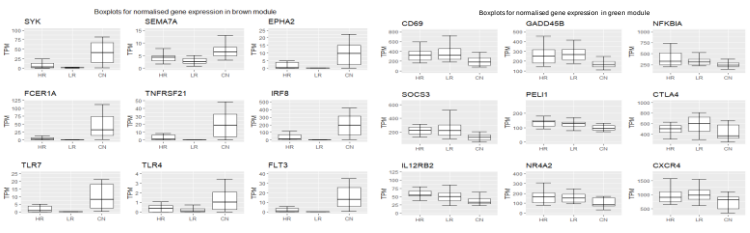
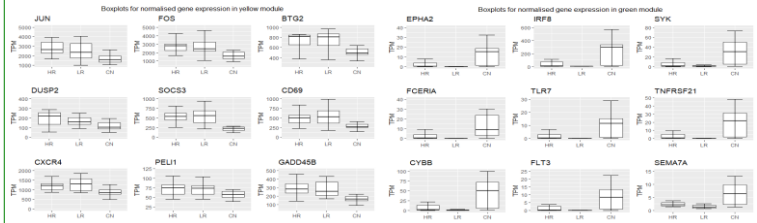


Figure 4: Heatmap of gene expression of hub genes in green module in NR (orange bar), R (blue bar) and CN (purple bar). **Figure 5:** Boxplots of normalised gene expression for selected genes in NR, R and CN in brown and green modules.



Th1 cells 11 modules were identified. The yellow module was associated with R vs CN and NR vs CN and showed genes and hub genes involved in T cell activation and genes that amplify it. Showing Th1 cells are activated in PBC, irrespective of response status. Green module was negatively correlated with R vs CN and showed genes and hub genes which were stimulatory. Suggesting stronger regulation of Th1 cells in UDCA responsive PBC.

Figure 3: Heatmap of gene expression of hub genes in green module in NR (orange bar), R (blue bar) and CN (purple bar). Figure 5. Boxplots of normalised gene expression for selected genes in NR, R and CN in yellow and green modules.



B cells identified 10 modules. Black module showed strong correlation with R vs CN and included genes involved in protein synthesis and B cell activation. Highlighting activation of B cells in UDCA response PBC

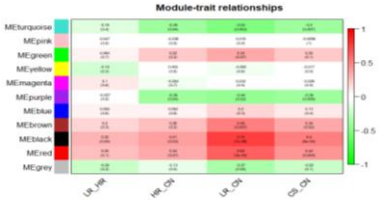


Figure 6. B cells. Module trait relationship table.

Th17 identified 11 modules, of which red and purple were associated with one or more traits. Red module was correlated with R vs CN and NR vs CN with genes and hub genes involved in T cell activation. Implying Th17 cells are activated in all PBC patients, irrespective of UDCA response.

Figure 7. Th17 cells. Module trait relationship table.

R vs NS comparison: Monocytes DEGs showed downregulation in R emphasising that these cells might be suppressed in well-controlled PBC patients. Th1 cells and Treg cells DEGs in R showed downregulation of genes such as IRF8, FLT3 and SYK suggesting a stronger regulation of Th1 cells in well controlled disease.

Conclusion: Our findings suggest monocytes are activated and proinflammatory in NR. Th1 cells are activated in all PBC cases irrespective of UDCA response. Treg cells have increased activity and also greater regulation in R compared to NR. We observed greater activation of B cells in R. Our results suggest that the innate and adaptive immune responses are contained in R but less so in NR.