Glycolysis and oxidative stress related redox pathway upregulation along the gut-liver axis by gut microbial perturbation and host response during giardiasis in C57BL/6J mouse model

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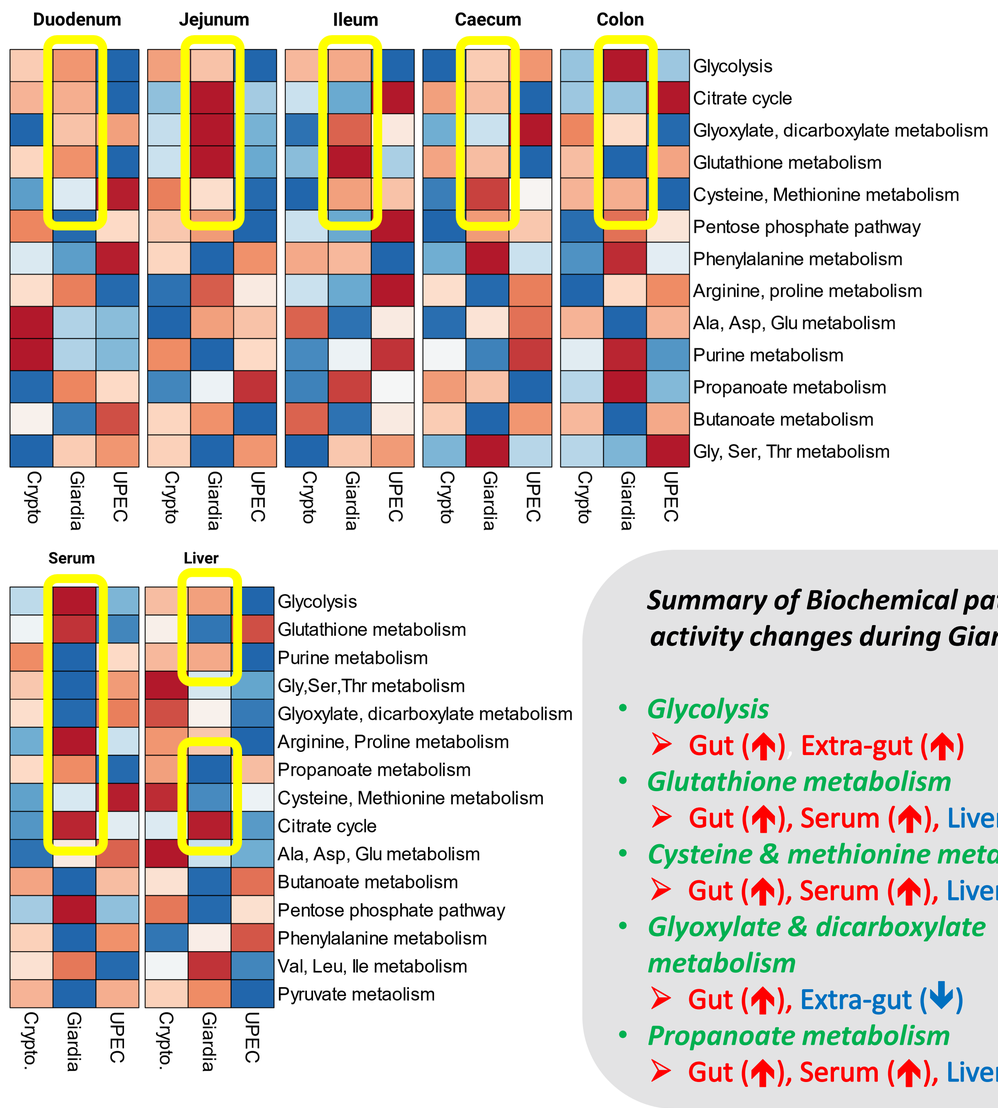
Apicomplexan infections such as giardiasis and cryptosporidiosis negatively affect a considerable number of human and commercial livestock. Such infectious events shows impact at various levels. To understand these biological changes, here we conducted we utilised integrated 16S rRNA genomics-metabolomics, and proteomics-metabolomics approaches on a C57BL/6J mouse model during giardiasis, with respect to cryptosporidiosis and Uropathogenic *E. coli* (UPEC) infection

Material and Methodology

- Host-parasite biochemical interaction during giardiasis with respect to cryptosporidiosis and UPEC were assessed using C57BL/6J, BALB/c and Swiss mice (n = 3).
- Microbial, protein and metabolic extractions from gut sections(Duodenum, jejunum, ileum, cecum, colon and faecal samples), blood serum and liver were conducted.
- V3 and V4 regions of 16S rRNA amplified and sequenced on Illumina MiSeq platform. The data was analysed using QIIME2 Pipeline
- Proteome data anlyzed against Uniprot *Mus musculus* protein database (UP000000589).
- Metabolomic analysis was performed on Agilent 7890B gas chromatography system with mass spectrometry.
- Taxanomy – to – phenotype mapping of gut microbiome and metabolome was performed using Burrito and MIMOSA2 analysis.
- Proteomic-metabolomic integration and networking through 'Joint-pathway analysis' tool (Metaboanalyst 5.0) and Paintomics 3.0 web toolbox.



Summary of Biochemical pathway activity changes during Giardiasis

- ***Glycolysis***
  - ➢ Gut (↑), Extra-gut (↑)
- ***Glutathione metabolism***
  - ➢ Gut (↑), Serum (↑), Liver (↓)
- ***Cysteine & methionine metabolism***
  - ➢ Gut (↑), Serum (↑), Liver (↓)
- ***Glyoxylate & dicarboxylate metabolism***
  - ➢ Gut (↑), Extra-gut (↓)
- ***Propanoate metabolism***
  - ➢ Gut (↑), Serum (↑), Liver (↓)

Figure 4: The heatmap represents Log2Fold change-based relative upregulation (red) and downregulation (blue) of individual pathways in the mice with infected gut with respect to the uninfected mice.

Results

Mouse strain selection

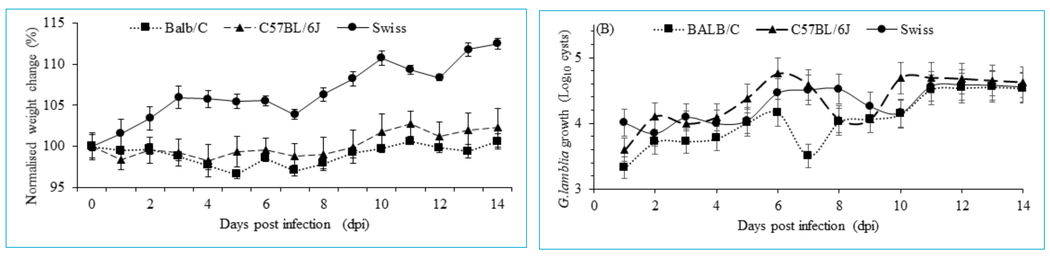


Figure 1: Growth of (A) Mice during the period of infection and (B) G. lamblia count during the period of infection. On 10 – 11 dpi, the G. lamblia cyst count showed a second peak before stabilising. Based on the outputs of both mice weights, G. lamblia cyst count and response to the infection, C57BL/6J strain was selected, with the follow-up main study shortened to 10 dpi.

Gut and extra-gut metabolism: Multi-omics integration

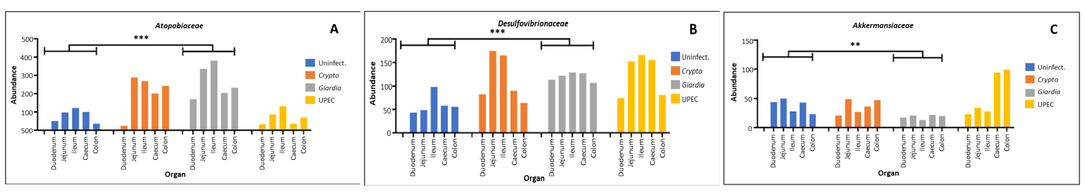


Figure 2: The spread of bacterial species which showed the most significant changes during giardiasis with respect to eukaryotic (cryptosporidiosis) and prokaryotic (UPEC) infections with significance of $p \leq 0.01$ (***) and 0.05 (**), respectively.

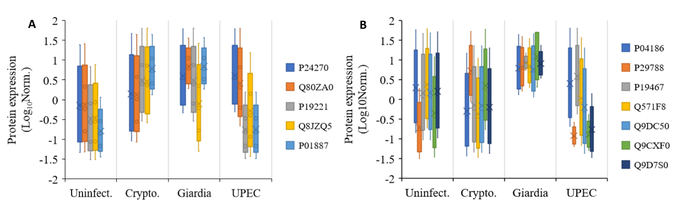


Figure 3: Log_{10}normalised expression of proteins from **(A)** Serum and **(B)** Liver which were observed to be involved in cellular protection as an extra-intestinal effect of various gut infections in the mouse model. Note: The protein identifiers refer to the Uniprot IDs.

Conclusions

- Proteome-metabolome analyses indicated 12 and 16 key pathways significantly altered throughout gut and liver, respectively, during giardiasis with respect to other infections.
- Metabolomics-16S rRNA genetics integration indicated the populations of 3 bacterial families of *Autopobiaceae* (Up), *Desulfovibrionaceae* (Up) and *Akkermanasiaceae* (Down) to be most significantly affected across the gut during giardiasis, causing upregulated glycolysis and short-chained fatty acid (SCFA) metabolism.
- Oxidative stress triggered the upregulation of glutathione metabolism in small intestine and liver, indicating towards an activation of redox pathway, as a stress response mechanism.
- Our observations indicate towards the capability of multiomics integration to ascertain a comprehensive understanding of host-parasite interaction throughout the gut, and the previously unreported effects of these interactions on gut-liver axis.
- The outputs of this study will potentially aid towards developing of precision medicine for gut infections.

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FOR FURTHER INFORMATION

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REFERENCES

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