

Single-cell gene expression links SARS-CoV-2 infection and gut serotonin

We read with great interest the paper by Ha *et al*¹ demonstrating that circulating levels of serotonin (5-hydroxytryptamine, 5-HT) are increased in COVID-19 and correlate with disease severity and gastrointestinal symptoms such as diarrhoea. Another recent paper by Lin *et al*² in this journal demonstrated that diarrhoea is the most common GI symptom in patients with COVID-19. Almost all 5-HT in our body is produced by enterochromaffin (EC) cells within the epithelium of the GI tract, which constitute approximately half of all enteroendocrine (EE) cells. Gut-derived 5-HT modulates gut peristalsis and exacerbates inflammatory responses by acting as a chemotactic molecule for various immune cells and by triggering cytokine release.³ While most gut epithelial cell types are susceptible to SARS-CoV-2 infection, EE cells have the greatest proportion of cells infected at 12 hours after viral exposure.⁴ In addition, the use of selective serotonin reuptake inhibitors (SSRI), normally prescribed to treat mental health conditions such as depression, is reported to reduce COVID-19 severity in humans.⁵ The GI tract is a route of SARS-CoV-2;⁶ however, its unknown if EC cells have any specific capacity for infection that would explain the increased 5-HT in patients with COVID-19, or the SSRI treatment efficacy reported. We, therefore, examined (see online supplemental file 1) the transcriptomes of cells lining the gut wall⁷ for expression of genes associated with SARS-CoV-2 infection, with a focus on EE cell subtypes.

Our focus was on gene expression for proteins implicated or known to be involved as COVID-19 receptors for efficient cell entry; ACE2, BSG and NRP1, associated proteins involved in intracellular trafficking and breakdown; TMPRSS2, FURIN and CTSB, and proteins associated with viral protection; LY6E, IFITM1-3 and IFNAR1-2. We identified that the genes encoding for all of these proteins are expressed within the intestinal epithelium (figure 1). Of the known COVID-19 receptors, *Ace2* and *Bsg* genes are highly expressed in all epithelial cell types. However, the more recently identified receptor, NRP1, is expressed exclusively in hormone-producing EE cells at the gene level.

To examine this further, we determined which subtypes of EE cells express

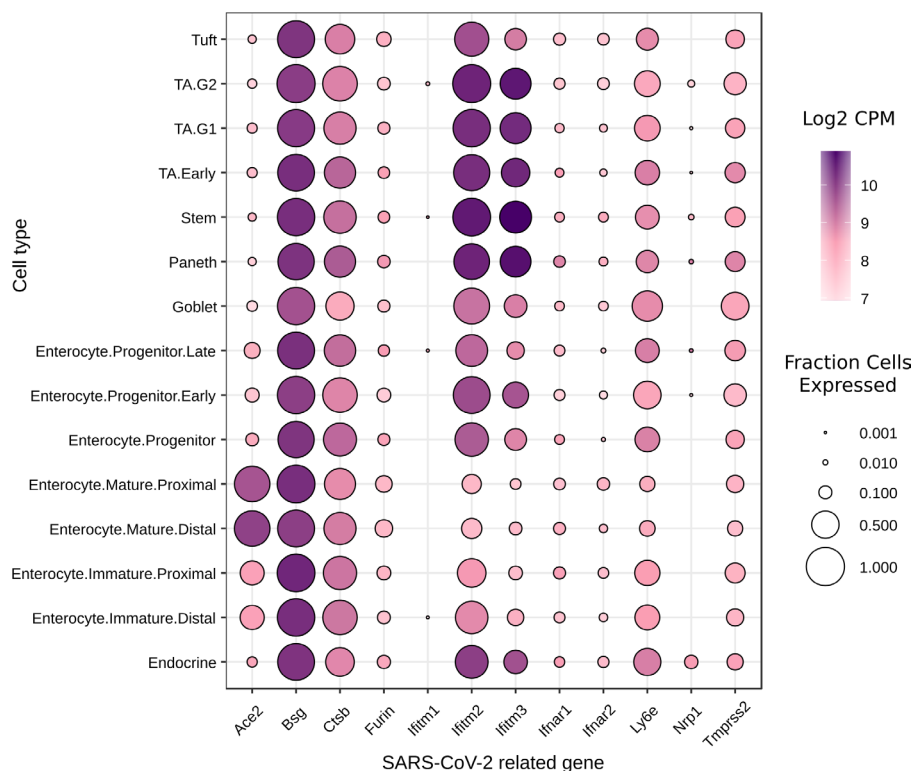


Figure 1 Absolute expression of SARS-CoV-2-related genes in different cell types from the mouse small intestinal epithelium.⁷ Data are shown as colour scale of Log2 of mean copies per million (CPM), with circle size indicating fraction of each cell type expressing the gene.

Nrp1 (figure 2). We focused on the major EE cell types containing cholecystokinin, glucagon-like peptide-1, ghrelin, neurotensin, somatostatin and 5-HT (figure 2A). EC cells that express tryptophan hydroxylase 1 (*Tph1*), the rate-limiting enzyme for non-neuronal 5-HT synthesis, are the primary cell type in the gut wall expressing *Nrp1*, indicating these cells may be a route of infection and disease pathogenesis. We then focused solely on EC cell gene expression using a second RNA-seq database⁸ and found that while all COVID-19-related genes are expressed in EC cells, *Nrp1* has the greatest enrichment of expression of all

these, of approximately 45-fold greater expression in EC cells than in non-EC epithelial cells (figure 2B). Subsequent examination of published work identifies that NRP1 protein expression is highly colocalised in the gastrointestinal wall with cells that express chromogranin-A, a marker of EC cells.⁹

Cells expressing NRP1, ACE2 and TMPRSS2 have a >3-fold increase in SARS-CoV-2 infection compared with ACE2 and TMPRSS2 alone.¹⁰ Our data demonstrate that EC cells are the only gut cell type that expresses significant levels of these three SARS-CoV-2-related genes. This, therefore, provides a link between

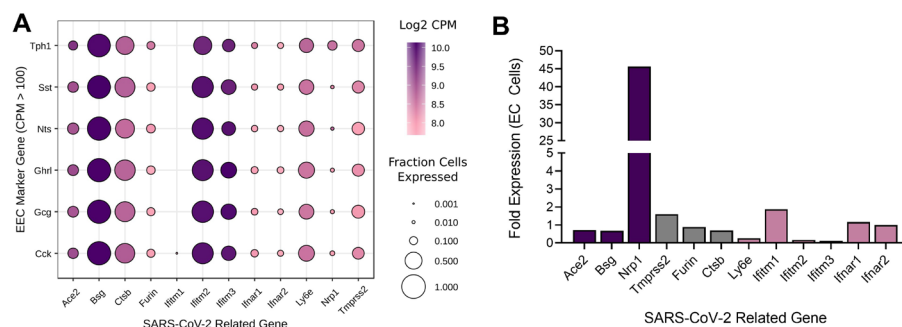


Figure 2 Absolute expression levels of SARS-CoV-2 related genes in different enteroendocrine cell (EEC) types from the mouse small intestinal epithelium.⁷ Data are shown as colour scale of Log2 of mean copies per million (CPM), with circle size indicating fraction of each cell type expressing the gene.

EC cells and the increased diarrhoea,² circulating 5-HT,¹ and efficacy of SSRIs⁵ that are reported in COVID-19. Experiments investigating SARS-CoV-2 infectivity in the absence of gut-derived 5-HT would provide further evidence of this link.

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Contributors AMM, LAJ and DJK developed the concept; MR and RE performed bioinformatic analysis; MR and AMM made the figures; AMM, LAJ, DT, RAC, CA and DJK reviewed the literature and wrote the drafts; all authors read and approved the final manuscript for submission.

Funding Supported by grants from the Australian Research Council (DE220100403 to AMM).

Competing interests None declared.

Patient consent for publication Not applicable.

Ethics approval Not applicable.

Provenance and peer review Not commissioned; internally peer reviewed.

Supplemental material This content has been supplied by the author(s). It has not been vetted by

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► Additional supplemental material is published online only. To view, please visit the journal online (<http://dx.doi.org/10.1136/gutjnl-2022-328262>).



To cite Martin AM, Roach M, Jones LA, *et al.* *Gut* Epub ahead of print: [please include Day Month Year]. doi:10.1136/gutjnl-2022-328262

Received 11 July 2022
Accepted 12 August 2022

Gut 2022;**0**:1–2. doi:10.1136/gutjnl-2022-328262

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REFERENCES

- Ha S, Jin B, Clemmensen B, *et al.* Serotonin is elevated in COVID-19-associated diarrhoea. *Gut* 2021;70:2015–7.
- Lin L, Jiang X, Zhang Z, *et al.* Gastrointestinal symptoms of 95 cases with SARS-CoV-2 infection. *Gut* 2020;69:997–1001.
- Yabut JM, Crane JD, Green AE, *et al.* Emerging roles for serotonin in regulating metabolism: new implications for an ancient molecule. *Endocr Rev* 2019;40:1092–107.
- Triana S, Metz-Zumaran C, Ramirez C, *et al.* Single-cell analyses reveal SARS-CoV-2 interference with intrinsic immune response in the human gut. *Mol Syst Biol* 2021;17:e10232.
- Reis G, Dos Santos Moreira-Silva EA, Silva DCM, *et al.* Effect of early treatment with fluvoxamine on risk of emergency care and hospitalisation among patients with COVID-19: the together randomised, platform clinical trial. *Lancet Glob Health* 2022;10:e42–51.
- Zhang H, Kang Z, Gong H, *et al.* Digestive system is a potential route of COVID-19: an analysis of single-cell coexpression pattern of key proteins in viral entry process. *Gut* 2020;69:1010–8.
- Haber AL, Biton M, Rogel N, *et al.* A single-cell survey of the small intestinal epithelium. *Nature* 2017;551:333–9.
- Bellono NW, Bayrer JR, Leitch DB, *et al.* Enterochromaffin cells are gut Chemosensors that couple to sensory neural pathways. *Cell* 2017;170:185–98.
- Yu DCW, Bury JP, Tiernan J, *et al.* Short-chain fatty acid level and field cancerization show opposing associations with enteroendocrine cell number and neuropeptide expression in patients with colorectal adenoma. *Mol Cancer* 2011;10:27.
- Cantuti-Castelvetri L, Ojha R, Pedro LD, *et al.* Neuropeptide-1 facilitates SARS-CoV-2 cell entry and infectivity. *Science* 2020;370:856–60.