

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

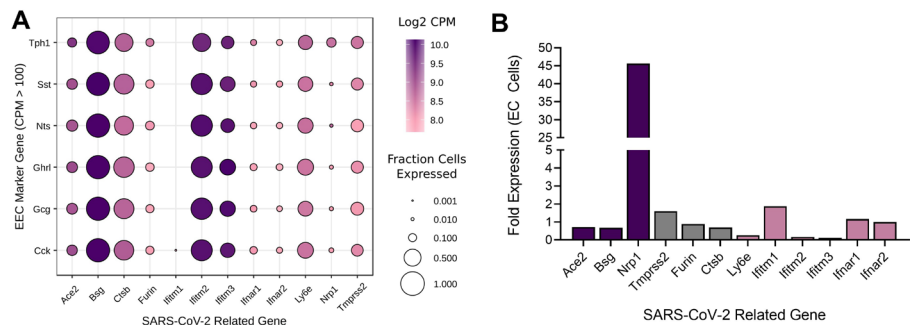
## Single-cell gene expression links SARS-CoV-2 infection and gut serotonin

We read with great interest the paper by Ha *et al*<sup>1</sup> 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*<sup>2</sup> 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.<sup>3</sup> 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.<sup>4</sup> 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.<sup>5</sup> The GI tract is a route of SARS-CoV-2;<sup>6</sup> however, it is 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 wall<sup>7</sup> 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.



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

To examine this further, we determined which subtypes of EE cells express *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 database<sup>8</sup> 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.<sup>9</sup>

Cells expressing NRP1, ACE2 and TMPRSS2 have a >3-fold increase in SARS-CoV-2 infection compared with ACE2 and TMPRSS2 alone.<sup>10</sup> 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 EC cells and the increased diarrhoea,<sup>2</sup> circulating 5-HT,<sup>1</sup> and efficacy of SSRIs<sup>5</sup> 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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