Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

agree with the need for further high-quality and prospective data to help improve our care of these patients.

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Conflicts of Interest
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Most current article

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TNFα-Antagonist Use and Mucosal Inflammation Are Associated with Increased Intestinal Expression of SARS-CoV-2 Host Protease *TMPRSS2* in Patients with Inflammatory Bowel Disease

Dear Editors:

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) viral transfection is dependent on angiotensin-converting enzyme 2 (ACE2) and transmembrane serine protease 2 (TMPRSS2), and increased intestinal receptor expression might support viral replication.[1–3] Therefore, we read with great interest the work by Krzysztof et al, reporting that anatomic location, intestinal inflammation, and age are key determinants of intestinal expression of *ACE2* and *TMPRSS2* in patients with inflammatory bowel disease (IBD).[1] However, several additional questions remain as the authors themselves also describe. It is important to understand the effects of immunomodulating drugs on *ACE2* and *TMPRSS2* expression, and the cell-type–specific context in which these genes are expressed.[1] In addition, it is important to understand the genetic determinants of intestinal *ACE2* and *TMPRSS2* expression. To address these remaining questions, we queried host genetic data and RNA sequencing data of intestinal biopsies from patients with IBD. We independently confirm that anatomic location, intestinal inflammation, and age are determinants of intestinal *ACE2* and *TMPRSS2* expression. Furthermore, we demonstrate that immunomodulating drugs are associated with increased intestinal *TMPRSS2* expression, and highlight the cell-type–specific context of *ACE2* and *TMPRSS2* expression.

We analyzed bulk RNA sequencing data of 92 ileal and 199 colonic snap frozen mucosal biopsies from 168 patients with IBD, as a part of the 1000IBD project.[4] Genotypes were obtained using both imputed Global Screening Array data and whole exome sequencing. We analyzed clinical data regarding the use of immunomodulating drugs, age, sex, diagnosis (Crohn's disease, ulcerative colitis, or unclassified IBD) and body mass index (BMI). Multivariate linear mixed regression analyses were performed to assess the effects of these clinical factors on the intestinal gene expression levels of *ACE2* and *TMPRSS2* (R v.3.6.0). In addition, we assessed the effects of host genetic variation on gene expression (*cis*-expression quantitative trait loci). All of these clinical factors, as well as sequencing batch, were included as covariates. We performed deconvolution analyses on bulk RNA sequencing data to assess cell-type–specific expression of *ACE2* and *TMPRSS2*. We validated this approach using single-cell RNA sequencing data from an independent set of 18 colonic biopsies from 11 patients with ulcerative colitis (unpublished data; 1000IBD cohort; 2020).

First, we replicated the effects of anatomic location, intestinal inflammation, and age on intestinal expression of *ACE2* and *TMPRSS2*. The expression of *ACE2* was higher, and *TMPRSS2* was lower in ileum compared to colon ($P < 2.2 \times 10^{-16}$). Moreover, the expression of *ACE2* was lower and the expression of *TMPRSS2* was higher in inflamed ileum compared with a noninflamed ileum, independent of medication use, age, sex, diagnosis, and BMI ($P = 4.4 \times 10^{-6}$ and $P = 8.6 \times 10^{-8}$, respectively). Ileal *TMPRSS2* expression was associated with increasing age ($P = .04$). Furthermore, we found increased *TMPRSS2* expression in intestinal (ileal and colonic) biopsies of male patients ($P = .02$).

Second, we studied the effects of immunomodulating drugs on *ACE2* and *TMPRSS2* expression in intestinal biopsies. We found increased *TMPRSS2* expression in ileal biopsies of patients using tumor necrosis factor alpha antagonists ($P = 8.8 \times 10^{-6}$), independent of intestinal inflammation, age, sex, diagnosis, and BMI. Because aminosalicylates were only used in the context of ulcerative colitis, we assessed the influence of aminosalicylates in colonic tissue only, and observed an increased *TMPRSS2* expression ($P = .02$). The use of thiopurines or steroids was not associated with differential expression of *ACE2* or *TMPRSS2.* Furthermore, host genetic variation was not associated with differential expression of *ACE2* or *TMPRSS2*.

Third, we interrogated the cell-type–specific contexts of intestinal *ACE2* and *TMPRSS2* expression. Using bulk RNA sequencing data, we quantified cell-type proportions and observed that an enrichment of epithelial cells is associated with increased expression of *ACE2* and *TMPRSS2* in both ileum and colon (all $P < .004$). Using single-cell RNA sequencing data from colonic biopsies, we observed that *TMPRSS2* is mainly expressed by absorptive enterocytes, and that intestinal inflammation is associated with increased *TMPRSS2* expression within absorptive enterocytes ($P = 3.2 \times 10^{-22}$). *ACE2* was also primarily expressed by absorptive enterocytes, but expression was not affected by intestinal inflammation.

In conclusion, ACE2 and TMPRSS2 are key proteins for cellular entry of SARS-CoV-2, and are highly expressed in the intestinal mucosa. Next to intestinal inflammation, age, sex, and anatomic location, the use of tumor necrosis factor alpha antagonists and aminosalicylates influence intestinal expression of *TMPRSS2* as well. We demonstrate that intestinal inflammation is associated with an increased expression of *TMPRSS2* in absorptive enterocytes, suggesting that the increased expression is not merely an effect of change in cellular composition during inflammation. Altered intestinal expression could render patients with IBD particularly susceptible to COVID-19 and absorptive enterocytes could provide targets for interventional studies. Indeed, clinical studies are needed to monitor the impact of COVID-19 on patients with IBD.

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Conflicts of interest
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Reply. We read with great interest the letter by Bangma et al, who corroborated our previous findings, and extended these by demonstrating increased expression of *TMPRSS2* in the ileal mucosa of patients with inflammatory bowel disease (IBD) receiving antagonists of tumor necrosis factor α (TNFα). Bangma et al also demonstrate that 5-aminosalicylates (5-ASA) use may be associated with increased colonic *TMPRSS2* expression.

The translational implications of these emerging data in terms of the prediction of susceptibility and severity of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection in IBD are now key issues, which remain to be established. The observed effect of anti-TNFα therapy on *TMPRSS2* expression in Bangma's report are difficult to reconcile with conclusions from a recent IBD meta-analysis,[1] wherein anti-TNFα agents associated with a 3-fold decrease in the risk of hospitalization for coronavirus disease 2019 (COVID-19). Further investigation is clearly required, focusing both on the gut as well as extraintestinal effects, notably in the respiratory system. For example, can it be that any "negative" effects of TMPRSS2-related facilitation of viral entry in the intestine are more than offset by the effects of TNF blockade in curtailing inflammation in the lungs? This question and related uncertainties in assessing the translational relevance will need to be answered by exploring gene function and expression in parallel with clinical observations, including outcomes from ongoing trials of infliximab in COVID-19 (NCT04425538, NCT04593940).

The potential contribution of 5-ASA therapy to the course of COVID-19 is also proving to be complex to unravel. The meta-analysis revealed an increased risk of COVID-19 hospitalization in IBD related to 5-ASA therapy (by 59%).[1] In the SECURE-IBD registry, severe COVID-19 was more frequent in patients receiving 5-ASA (adjusted odds ratio of 1.7).[2] In discussing the initial SECURE-IBD report,[3] Magro et al pointed out[4] that binding of peroxisome proliferator-activated receptor gamma by 5-ASA could induce *ACE2* and inhibit *TMPRSS2* expression. Here, Bangma et al, to the contrary, indicate increased colonic *TMPRSS2* expression in patients with ulcerative colitis receiving aminosalicylates. Again, we suggest that consideration of *TMPRSS2* expression in the ileum might not be key for the systemic course of COVID-19.

We suggest that it is important to consider the implications and pathogenesis of multiorgan involvement in COVID-