Brief Communication

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Quantitative Proteomic Analysis of the Expression of SARS-CoV-2 Receptors in the Gut of Patients with Chronic Enterocolitis

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The cellular entry of severe respiratory syndrome coronavirus-2 (SARS-CoV-2) is mediated by interaction with the human angiotensin-converting enzyme 2 (ACE2), a receptor that is expressed on both lung and intestinal epithelial cells. We performed a quantitative proteomic analysis to investigate the expression of possible receptors for SARS-CoV-2 in the intestinal mucosa of 23 patients with chronic colitis. ACE2 expression was low and remained unaltered in the gut of patients with ulcerative colitis (UC), Crohn's disease (CD), intestinal Behćet's disease (BD), and intestinal tuberculosis (TB), when compared with that of healthy individuals. Additionally, the expression levels of some probable co-receptors, including dipeptidyl peptidase 4 (DPP4), aminopeptidase N (AMPN), and glutamyl aminopeptidase (AMPE), were unchanged in the affected UC, CD, intestinal BD, and intestinal TB colon mucosa samples. In conclusion, gut inflammation associated with chronic colitis does not mediate a further increase in the cellular entry of SARS-CoV-2.

Key Words: Coronavirus, SARS-CoV-2, COVID-19, angiotensin converting enzyme 2, proteomics

The coronavirus disease 2019 (COVID-19) pandemic, caused by severe respiratory syndrome coronavirus-2 (SARS-CoV-2), is ongoing due to a rapid increase in the number of cases outside Wuhan, China.[1] The high-affinity interaction of SARS-CoV-2 with the human angiotensin-converting enzyme 2 (ACE2) receptor has been shown to mediate the entry of the virus into the cell.[2] ACE2 is not only expressed in lung epithelial cells, but also in colon and terminal ileum epithelial cells.[3] Accordingly, gastrointestinal manifestations, including diarrhea, nausea, and vomiting, were reported in 4% to 23.7% of COVID-19 patients.[1,4] Additionally, continuous positive detection of SARS-CoV-2 RNA in feces has been demonstrated in a previous study, suggesting that fecal-oral transmission is as an alternative route of infection.[5] Furthermore, dipeptidyl peptidase 4 (DPP4), aminopeptidase N (AMPN), and glutamyl aminopeptidase (AMPE) have been suggested to be probable SARS-CoV-2 co-receptors, in conjunction with ACE.[6]

A few studies have investigated the susceptibility of COVID-19 infections in patients with chronic colitis. Therefore, we performed a quantitative proteomic analysis associated with COVID-19 utilizing tissue samples of patients with chronic colitis, including ulcerative colitis (UC), Crohn's disease (CD), intestinal Behćet's disease (BD), and intestinal tuberculosis (TB). Subsequently, the protein expression levels in these patients were compared with those of healthy controls.

Chronic colitis patients, including those with UC (n=7), CD (n=7), intestinal BD (n=7), and intestinal TB (n=2), along with

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healthy controls (n=7), were recruited between November 2006 and September 2019 at the IBD Clinic at Severance Hospital in Seoul, Korea. The colon mucosa tissue samples were obtained using colonoscopy guided biopsy of the affected bowel with chronic colitis. Conversely, the normal colon mucosa tissue samples were obtained from healthy individuals. The tissue and serum samples were preserved at -80°C. This study was approved by the Institutional Review Board of Yonsei University College of Medicine (IRB No: 2012-0039-030).

For proteomic analysis, the colon mucosa tissue samples were lysed and labeled with tandem mass tag (TMT, Thermo Scientific, San Jose, CA, USA) reagents in accordance with the manufacturer's instructions. The labeled peptide samples were pooled into a new vial and dried in a SpeedVac (Thermo Scientific). Various methodologies, including strong cation exchange fraction, liquid chromatography (LC)-mass spectrometry (MS), and a database search, were performed by Poochon Scientific (Frederick, MD, USA). MS raw data files were searched against the human protein sequence databases obtained from the NCBI website for the database search, which was conducted using Proteome Discoverer 1.4 software (Thermo Scientific) based on the SEQUEST and percolator algorithms.

The false positive discovery rate was set to 5%. The resulting Proteome Discoverer report from Poochon Scientific contained the assembled proteins, along with pertinent data regarding the peptide sequences and spectrum match counts (PSM#), as well as the TMT-tag based quantification ratio. Unpaired Student's t-test was used to compare the differences in expression between the chronic colitis patients and the healthy controls. A two-sided p value of < 0.05 was considered to be statistically significant.

The colon mucosa tissue samples from HCs and patients with UC, CD, intestinal BD, and intestinal TB were collected and analyzed using the TMT/LC-MS/MS-based proteomic approach. We identified 5137 proteins from the TMT set (2 HC vs. 2 CD vs. 2 UC vs. 2 BD vs. 2 TB) and 6154 proteins from a combination of two TMT sets (5 HC vs. 5 CD vs. 5 UC vs. 5 BD). ACE2 was detected only in the first TMT set, whereas, DPP4, AMPN, and AMPE were detected in all TMT sets.

ACE2 expression levels were 1.18-fold higher in UC, 0.97-fold lower in CD, 1.59-fold higher in intestinal BD, and 1.52-fold higher in intestinal TB, compared with expression levels in healthy controls. However, the observed changes in expression levels were not statistically significant (Table 1, Fig. 1A). Furthermore, DPP4, AMPN, and AMPE expression levels did not differ between chronic colitis patients and healthy controls (Table 1, Fig. 1B–D).

In the present proteomics analysis, we investigated the expression levels of possible SARS-CoV-2 receptors in the intestinal mucosa of chronic colitis patients. Unexpectedly, the expression levels of the SARS-CoV-2 receptor ACE2 were extremely low. Additionally, the quantitative analysis did not show elevated ACE expression levels in UC, CD, intestinal BD, and intestinal TB patients, compared with those in healthy controls. Other than ACE2, the expression levels of several other possible co-receptors, including DPP4, AMPN, and AMPE, were not increased in the affected UC, CD, intestinal BD, and intestinal TB colon mucosa samples.

A higher expression of ACE2 has been reported in the terminal ileum and colon tissues of IBD patients, compared to that in control patients, on the basis of immunohistochemical staining.[7] However, in agreement with our findings, no significant differences in ACE2 expression between IBD patients and healthy controls were observed in TMT-based shotgun proteomic studies, which utilized quantitative proteomics to conduct a high-throughput analysis of protein expression with enhanced accuracy, sensitivity, and reproducibility.[8,9] Moreover, a recent epidemiological study conducted by An, et al.[10] reported that SARS-CoV-2 infections were not detected in any of the 319 registered IBD patients in Wuhan, China. Hence, there is no evidence to suggest an increased risk of COVID-19 in IBD patients.

Although the proteomics analysis we applied here has strong aspects of accuracy and repeatability, there are a few limitations in this study. First, the sample size was relatively small to conclude that gut inflammation associated with chronic colitis would not mediate a further increase in the cellular entry of SARS-CoV-2 virus. Further investigation with more samples would be required to prove our hypothesis. Second, although ACE2 serves as a receptor for viral entry, some studies have shown that decreased ACE2 expression could portend a higher risk of developing severe acute respiratory disease, including lung injury. Imai, et al.[11] have revealed that ACE2 plays a protective role in severe lung injury in ACE2 knockout mice. Moreover, several studies have shown that although children have higher levels of ACE2 than adults, they have been shown to have fewer and less severe symptoms compared with adults.[12]

Table 1. Changes in Protein Expression in Chronic Colitis Patients

UniProt accession #	Protein name	UC/Con		CD/Con		Intestinal BD/Con		Intestinal TB/Con		Unique peptides/ Σ# PSMs
		FC	*p* value	FC	*p* value	FC	*p* value	FC	*p* value	
Q9BYF1	ACE2	1.18	0.739	0.97	0.142	1.59	0.325	1.52	0.397	1/1
P27487	DPP4	0.91	0.453	0.91	0.475	1.45	0.261	1.23	0.191	9/12
P15144	AMPN	0.88	0.531	0.98	0.910	1.76	0.256	1.41	0.178	26/67
Q07075	AMPE	1.02	0.969	1.15	0.769	3.56	0.282	2.00	0.223	6/7

UC, ulcerative colitis; CD, Crohn's colitis; BD, Behćet's disease; TB, tuberculosis; FC, fold change; Σ# PSMs, total numbers of identified peptide sequences; ACE2, angio-tensin-converting enzyme 2; DPP4, dipeptidyl peptidase 4; AMPN, aminopepti-dase N; AMPE, glutamyl aminopeptidase.

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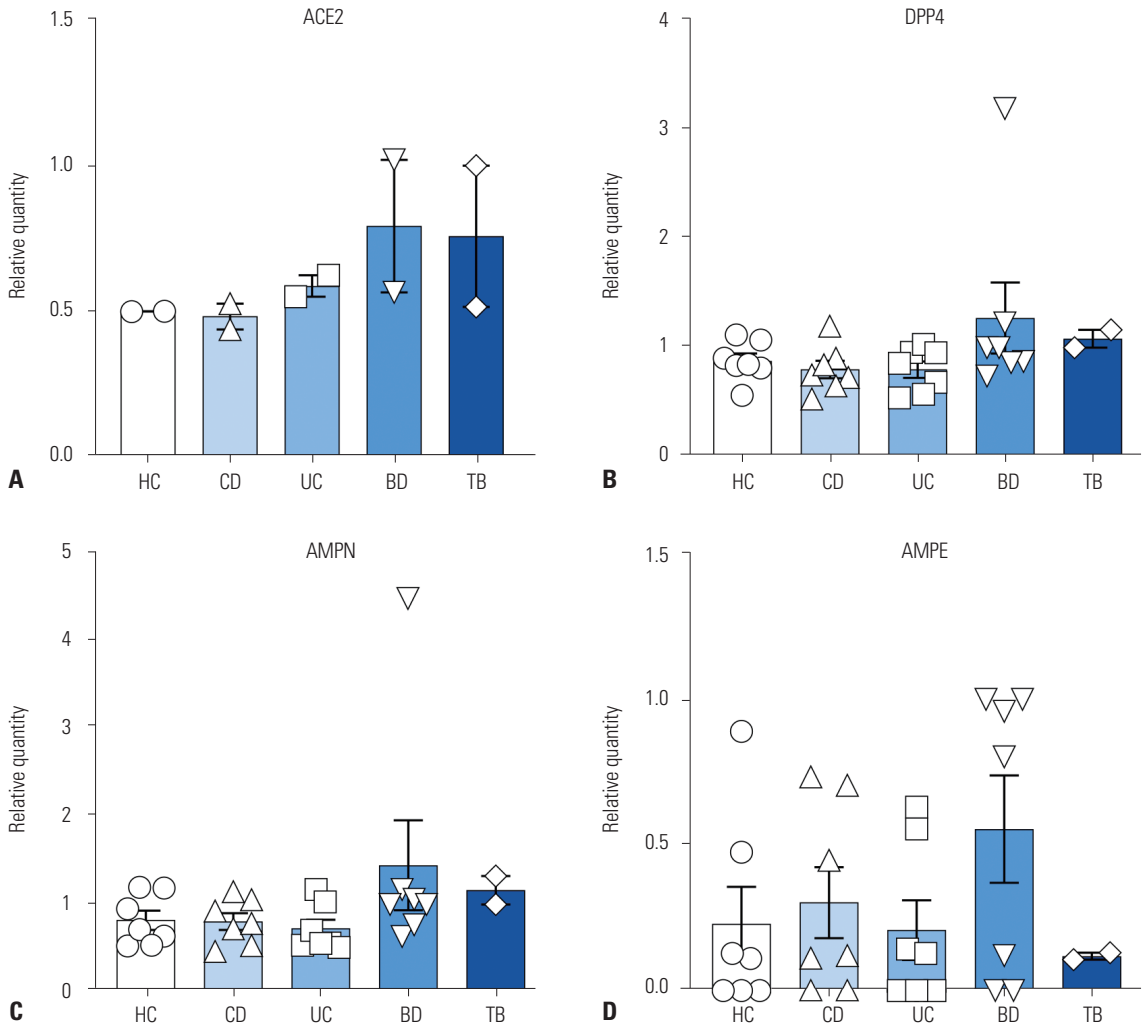

Fig. 1. Expression of SARS-CoV-2 receptor proteins in healthy controls (HC, open circle) and patients with Crohn's disease (CD, open triangle), ulcerative colitis (UC, open quadrangle), intestinal Behćet's disease (BD, open inverted triangle), and intestinal tuberculosis (TB, open diamond). (A) ACE2, (B) DPP4, (C) AMPN, and (D) AMPE. Data are representative of means±standard errors. ACE2, angio-tensin-converting enzyme 2; DPP4, dipeptidyl peptidase 4; AMPN, aminopepti-dase N; AMPE, glutamyl aminopeptidase.

In order to elucidate the functional role of ACE2 in specific diseases (e.g., chronic colitis), it is necessary to specifically examine it using specific disease models, such as human IBD mimicking animal colitis models.

Nevertheless, our data support the finding that gut inflammation associated with chronic colitis does not further potentiate the cellular entry of SARS-CoV-2.

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AUTHOR CONTRIBUTIONS

Conceptualization: Youn Wook Chung and Jae Hee Cheon. Data curation: Jihye Park, Daeun Jeong, Youn Wook Chung, and Da Hye Kim. Formal analysis: Jihye Park and Youn Wook Chung. Funding acquisition: Jae Hee Cheon and Ji-Hwan Ryu. Methodology: Youn Wook Chung. Project administration: Jae Hee Cheon and Ji-Hwan Ryu. Resources: Youn Wook Chung, Jae Hee Cheon, and Ji-Hwan Ryu. Software: Jihye Park and Youn Wook Chung. Supervision: Jae Hee Cheon and Ji-Hwan Ryu. Validation: Jihye Park and Youn Wook Chung. Visualization: Jihye Park and Youn Wook Chung. Writing—original draft: Jihye Park. Writing—review & editing: Youn Wook Chung, Jae Hee Cheon, and Ji-Hwan Ryu. Approval of final manuscript: all authors.

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