Expression profiles of the SARS-CoV-2 host invasion genes in nasopharyngeal and oropharyngeal swabs of COVID-19 patients

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ABSTRACT

We collect the nasopharyngeal and oropharyngeal swabs of 63 subjects with severe symptoms or contacts with COVID-19 confirmed cases to perform a pilot-study aimed to verify the “in situ” expression of SARS-CoV-2 host invasion genes (ACE2, TMPRSS2, PCSK3, EMILIN1, EMILIN2, MMRN1, MMRN2, DPP4). ACE2 (FC = +1.88, p < 0.05) and DPP4 (FC = +3, p < 0.01) genes showed a significant overexpression in COVID-19 patients. ACE2 and DPP4 expression levels had a good performance (AUC = 0.75, p < 0.001) in distinguishing COVID-19 patients from negative subjects. Interestingly, we found a significant positive association of ACE2 mRNA and PCSK3, EMILIN1, MMRN1 and MMRN2 expression and of DPP4 mRNA and EMILIN2 expression only in COVID-19 patients. Noteworthy, a subgroup of severe COVID-19 (n = 7) patients, showed significant high level of ACE2 mRNA and another subgroup of less severe COVID-19 patients (n = 6) significant raised DPP4 levels.

These results indicate that a group of SARS-CoV-2 host invasion genes are functionally related in COVID-19 patients and suggests that ACE2 and DPP4 expression level could act as genomic biomarkers. Moreover, at the best of our knowledge, this is the first study that shows an elevated DPP4 expression in naso- and oropharyngeal swabs of COVID-19 patient thus suggesting a functional role of DPP4 in SARS-CoV-2 infections.

1. Introduction

SARS-CoV-2 betacoronavirus is the cause of worldwide disease COVID-19 [1]. COVID-19 ranges from asymptomatic or mild sub-clinical symptoms to acute respiratory distress syndrome (ARDS). COVID-19 manifests differently depending on patients age, underlying health conditions, and gender [2]. A growing body of evidence indicates sex differences in the clinical outcome of COVID-19 [3,4]. It is known that although men and women have a similar sensitivity to SARS-CoV-2 infection, men are more prone to higher severity and mortality than women, regardless of age [5]. However, phenotypic variability of COVID-19 is a consequence also of the individual genetic variability, that can make certain subjects more susceptible or more resistant to viral infection and disease progression [6, 7]. Currently, no suitable vaccines are available for SARS-CoV-2, although there are ongoing clinical trials and also studies of treatments with monoclonal antibodies [8].

The identification of the molecular mechanisms underlying the development and progression of COVID-19 is crucial for identifying appropriate medical therapies to combat viral infection. The main mechanism of the entire process of infection include the action of different human proteins. ACE2 is the host receptor for the novel SARS-CoV-2 [9]. A recent expression profile of ACE2 RNA in lung at a single cell
resolution suggested that ACE2 expression is concentrated in alveolar type II (AT2) cells [10]. SARS-CoV-2 entry also depends on the activity of the TMPRSS2 protease previously detected in the nasal and bronchial epithelium [4, 6] where TMPRSS2 is co-expressed together with ACE2 [9, 11]. PCSK3/furin is one of the important proteases that facilitates viral invasion. In fact, it cleaves viral S protein and helps SARS-CoV-2 interconnection [11]. DPP4 is a type II transmembrane peptidase-4), GAPDH (glyceraldehyde-3-Phosphate Dehydrogenase), RPLP0 (ribosomal protein, large, P0), ACTB (β-Actin). Fw (Forward), Rev (Reverse). PCR (Polymerase Chain Reaction).

Table 1. Real-Time PCR primer sequences and amplification settings.

| Genes    | Accession number | Sequence (5'-3') | Annealing temperature (°C) | Size (bp) |
|----------|------------------|------------------|-----------------------------|-----------|
| ACE2     | NM_001371415     | Fw GGTGGGAGATGAAAAGCAGAG | 60                     | 150       |
|          |                  | Rev CTTGAACCTGGAATGTTAAAGG | 59                     |           |
| TMPRSS2  | NM_005656        | Fw GGGACATGGGGTCTGAGGAAT | 53                     | 137       |
|          |                  | Rev ACCAGGATCAGCTGAGTAC | 58                     |           |
| PCSK3    | NM_002569        | Fw GCATTGGAGGGGTGGCCAT | 61                     | 106       |
|          |                  | Rev CCCCCAGCTGGCCTGAGAT | 59                     |           |
| EMLIN1   | NM_007046        | Fw CCAAAGCATCATGTACCGC | 58                     | 110       |
|          |                  | Rev CACAGTATGCCCCTCCAA | 57                     |           |
| MMRN1    | NM_007351        | Fw TAGTCCAGATTTTTCACAGG | 57                     | 112       |
|          |                  | Rev TCCAGATTAAAAACGATAGG | 59                     |           |
| EMLIN2   | NM_032048        | Fw GTGTGCGGCAGGCTGCTG | 62                     | 102       |
|          |                  | Rev GGCACATGGGTACCTCCTAC | 59                     |           |
| MMRN2    | NM_024756        | Fw CCAGGCTCAGCTGACTGCC | 59                     | 111       |
|          |                  | Rev GGGACCACTTACGTCCTAC | 59                     |           |
| DPP4     | NM_001935        | Fw GAAGGTGTGATCTACTTTCATG | 59                     | 130       |
|          |                  | Rev CACAGCTCCAGCCTTTATC | 59                     |           |
| GAPDH    | NM_002046        | Fw AAGTGCCATGACCCAGTATTT | 59                     | 100       |
|          |                  | Rev TGAAGGGTCATGTAGGCA | 57                     |           |
| RPLP0    | NM_001002        | Fw ACCAGCTCTGCGAAGAACCT | 57                     | 198       |
|          |                  | Rev AAAAGGAGCTTCCTCTGGG | 59                     |           |
| ACTB     | NM_001101        | Fw ATTCGGAGAGATGACGAA | 59                     | 150       |
|          |                  | Rev GCTGATCCACATGTCGAGA | 59                     |           |

ACE2 and DPP4 genes resulted over-expressed in nasopharyngeal and oropharyngeal swabs of COVID-19 vs negative subjects. Noteworthy, a positive correlation among ACE2 mRNA and PCSK3, EMLIN1, MMRN1 and MMRN2 expression and between DPP4 mRNA and EMLIN2 expression only in COVID-19 patients suggest that a coordinated expression pattern of these genes is crucial for SARS-CoV-2 infection and these expression patterns may be useful for predictive diagnosis, prognosis and pathophysiology of COVID-19.

2. Materials and methods

2.1. Patients’ recruitment and sample collection

The nasopharyngeal and oropharyngeal swabs of 63 cases with acute respiratory symptoms or contacts with COVID-19 confirmed cases were collected from 20 March to 20 April 2020, during triage at the Emergency Room (ER) of Policlinico Tor Vergata, PTV (Rome, Italy). Patients’ swabs, collected in viral transport media (VTM), were referred to the Virology Unit of PTV for diagnosis. Residual positive (n = 35) and negative (n = 28) samples for SARS-CoV-2 were used for RNA expression analysis.

2.2. Diagnostic test of SARS-CoV-2

To detect the qualitative presence of SARS-CoV-2 viral nucleic acids, we used the Allplex™ 2019-nCoV Assay (Seqenoe Inc.) (http://www.see gene.com/upload/product/Allplex_2019_nCoV_performance_data.pdf). Nucleic acids were isolated and purified from 300 μL of specimen using an automated nucleic acid extraction system. The assay is designed to detect SARS-CoV-2 RdRP and N genes and E gene of all Sarbecovirus including SARS-CoV-2. Briefly, first line screening was done for E and RNase P (internal control) genes. Clinical samples positive for E gene (Ct
2.3. Gene expression studies

RNA extracts from nasopharyngeal and oropharyngeal swabs were evaluated by NanoDrop DS-11 (DeNovix) (5–40 ng/μl and 5–30 ng/μl, respectively for DNA and RNA) with a 260/280 ratio of ~1.8 for DNA and of ~2.0 for RNA. 100 ng of total RNA was used for retrotranscription into cDNA using the High Capacity cDNA Reverse Transcription Kit (Applied Biosystems, USA). We analyzed the expression of ACE2, TMPRSS2, PCSK3, EMILIN1, EMILIN2, MMRN1, MMRN2 and DPP4 genes. Real time PCR (qRT-PCR) has been performed using ABI7500 Fast Real-time PCR System (Life Technologies) and specific primer pairs (Table 1).

Primers have been designed and evaluated by using two different software: Primer BLAST (https://www.ncbi.nlm.nih.gov/tools/primer-blast/) and Tm Calculator (www.thermofisher.com). Primers were designed according the following criteria: 1. At least one primer must be designed at an exon-exon junction; 2. Primer pair must be separated by at least one intron on the corresponding genomic DNA; 3. PCR product size must be about 100–200 bp; 4. Common SNPs in the primers have been excluded after evaluation on Ensembl Genome Browser (https://wwwensembl.org/index.html); 5. Primers must amplify all the known isoform of each gene, when present. GAPDH, ACTB and RPLP0 genes were used for data normalization. The qRT-PCR expression analyses were performed in triplicate. Data analysis was performed using the comparative threshold cycle (Ct) method quantification (2–ΔΔCt method) (https://www.protocols.io/view/compative-ct-method-quantification-2-delta-delta-c).
most of the negative subjects (19/28) returned home or were kept under brief observation. Clinical data of the 63 subjects studied is summarized and reported in Table 2.

Most of the enrolled subjects were male (46/63; 73%). Age ranged between 20 and 92 years old (median age 61 years old). Twenty-seven of them are over 65 (18 men and 9 women). We clustered the 63 individuals in two groups: SARS-CoV-2 positive (COVID-19 patients, n = 35; 56%; 26 men and 9 women) and SARS-CoV-2 negative (negative subjects, n = 28; 44%; 20 men and 8 women). Age in COVID-19 patients ranged from 20 and 92 years old (median age 59 ± 16). Thirty-three out of 35 patients have been subjected to serological analysis, resulting positive for IgG only (94.3%). Eighteen out of 35 patients needed Intensive Care Unit (ICU) and, after clinical stabilization, two of them went back to the Infectious Diseases ward. Four out of 35 patients needed Intensive Care Unit (ICU) and, after clinical stabilization, two of them went back to the Infectious Diseases ward. Four out of 35 patients (all men, 11.4%) died of concurrent causes associated with SARS-CoV-2, on average, two weeks after hospitalization. The average length of hospitalization was 48 days; the most serious patient was hospitalized for just over two months in ICU. Twenty-six out of 35 patients (74.3%) were subjected to oxygen therapy at medium-low flows (FiO2 range 35–50%) with cycles of CPAP and BiPAP (FiO2 50–80%) for an average of 30 days of stay; two patients underwent invasive manoeuvres and therefore were intubated for an average of 14 days (FiO2 range 80–100%), while for seven subjects (20%) we were unable to find useful information about their ventilation (Table 2).

For the serum-blood analysis at the entrance, seven patients presented a dramatic neutrophilic condition (9.9 10³/μL ± 4.6, Table 2), twenty-three patients presented an important lymphopenia (0.9 10³/μL ± 0.4, Table 2). All subject presented no significant clinical CRP, LDH, IL-6 and TNF-α. One patient, later died, showed BNP value of 581 pg/mL (normal value <100 pg/mL) associated with a Troponin I hs value of 1543.4 ng/L (normal value < 34.2). In all the patients, we found an elevation of both fibrinogen (mean: 573.7 mg/dl, Table 2) and D-dimer (mean: 3083.4 ng/mL, Table 2).

Age in SARS-CoV-2 negative group ranged from 27 and 84 years old (median age 64 ± 17). All these subjects resulted negative for SARS-CoV-2 diagnostic test. Twenty-four patients (n = 24/28; 85.7%) passed through the ER, eight of which (33.3%) were kept under brief observation and subsequently discharged; four (16.7%) have been transferred to competent departments responsible for further treatments and assessments. None of these patients undergone oxygen-therapy at medium-high flows, except for cases of respiratory support via Venturi Mask (VMK; FiO2 range 24–31%).

### 3.2. Gene expression level in SARS-CoV-2 positive and negative nasopharyngeal and oropharyngeal swabs

We analyzed the expression level of the eight SARS-CoV-2 host invasion genes by qRT-PCR using specific primer pairs (Table 1) on cDNA from residual unidentified nasopharyngeal and oropharyngeal swabs of 35 COVID-19 patients and 28 patients negative for SARS-CoV-2 virus presence. ACE2 (FC = +1.88, p ≤ 0.05) and DPP4 (FC = +3.0; p < 0.01) showed a significant overexpression in nasopharyngeal and oropharyngeal swabs of COVID-19 vs negative patients (Figure 1, A-H). No significant differences were observed in the other genes, even if most of them resulted overexpressed in COVID-19 patients’ swabs (Figure 1, B-G).

Interestingly, seven COVID-19 patients (TP6, TP22, TP23, TP27, TP43, TP52, TP64; hereafter named as “ACE2h-COVID-19”) showed higher ACE2 mRNA level in nasopharyngeal and oropharyngeal swabs (Figure 2A). Accordingly, the COVID-19 patients’ group without these seven subjects show no difference in nasopharyngeal and oropharyngeal ACE2 expression when compared with negative individuals (Figure 2A).
Moreover, six COVID-19 patients (TP57, TP59, TP60, TP61, TP64, TP66; hereinafter referred to as “DPP4h-COVID-19 patients”) showed a higher DPP4 expression level in nasopharyngeal and oropharyngeal swabs when compared to negative subjects and other COVID-19 patients (Figure 2A). Furthermore, the COVID-19 patients’ group, without DPP4h-COVID-19 patients, shows no difference in nasopharyngeal and oropharyngeal DPP4 expression when compared to negative subjects (Figure 2A). Noteworthy, ACE2h-COVID-19 patients show DPP4 levels comparable to the other COVID-19 patients and to negative subjects (Figure 2B) and the same pattern is observed for DPP4h-COVID-19 patients (Figure 2B).

3.3. Clinical study of ACE2h-COVID-19 patients

ACE2h-COVID-19 patients showed comorbidities that included cardiovascular complications as smoking habits or high blood pressure and nephrolithiasis or chronic renal failure. Clinical data of ACE2h-COVID-19 patients are reported in Table 3.

One patient was diagnosed with laryngeal and prostate cancer (TP22). The youngest one had a chronic renal failure (TP52) and the woman an history of osteopenia. Three patients were hospitalized for about a month, one patient was hospitalized in Infectious Disease Unit then in ICU for 71 days, and the oldest one (TP22) died after 14 days of hospitalization. The average length of hospitalization was 35 days. Six out of patients were males (n = 6/7; 85.7%), age ranged from 40 and 92 years old (median age: 56 ± 16) (Table 3).

Clinical manifestations at the onset included fever (7/7), dry cough (4/7) and dyspnea (6/7); two patients referred also gastrointestinal disorders as vomiting, diarrhea and abdominal discomfort (TP27 and TP6). Computed tomography of the chest (CT) showed abnormal images and the typical signs of pneumonia as ground-glass opacity and local patchy shadowing. At the entrance, laboratory results showed a relative neutrophilia condition (3.3 10³/mL ± 0.9, Table 3). All seven patients presented low lymphocytes values (0.8 10³/mL ± 0.3, Table 3), indicating a lymphopenia. Six out seven patients showed elevated CRP (84.9 mg/L ± 39.7, Table 3) and higher LDH value (342.8 U/L ± 88.4, Table 3); three out of 7 patients higher levels of IL-6 (60.2 g/dl ± 38.7); and only two showed a TNF-α > 50 pg/ml (68.1 pg/ml ± 203.2). In all the patients we found an elevation of fibrinogen (588.6 mg/dL ± 198.1, Table 3), and all but two presented high D-dimer values (1370.4 ng/mL ± 364.5, Table 3). The oldest patient (TP22), later died, showed Troponin I value of 106.3 ng/L (normal values < 34.2 ng/L).

Due to a severe deficiency of oxygen in the blood (70.7 mmHg ± 11.8), all patients but one (TP64) were treated with oxygen therapy, in particular with mechanical with medium-high flows (FiO₂ range 50–60%) with cycles of CPAP (FiO₂ 60%) and BLB (FiO₂ 80%) for an average of 10 and 19 days of stay, respectively. Two patients were intubated. According to the clinical records, six out of 7 patients compared to the other COVID-
19 patients, had a more severe onset clinical course. The clinical course has been characterized by a worse respiratory outcome. As a matter of fact, all these patients have been treated with medium high flows oxygen therapy: four of them with a FiO2% range between 50-60% (TP27, TP6, TP23 and TP52), one with a FiO2% range between 50-80% (TP43), and the oldest one with a range of FiO2% between 80-100% (TP22).

### 3.4. Clinical study of DPP4h-COVID-19 patients

DPP4h-COVID-19 patients showed comorbidities that included high blood pressure (TP57, TP64 and TP66) and osteopenia (TP64). Their clinical data are reported in Table 4.

The outcome of these six patients was characterized by a better course than those of the ACE2h-COVID-19 patients group. Five were hospital-
ized in the Infectious Diseases Unit ward, only one in the Respiratory Diseases ward. Half of DPP4h-COVID-19 patients were male (median age: 71 ± 15). The women age ranged from 52 and 84 years old (median age: 68 ± 16). Interestingly, one of them (TP64) also showed higher ACE2 mRNA level in nasopharyngeal and oropharyngeal swabs (Table 3 and Table 4). Clinical manifestation at the onset include only fever (6/6). No one presented dry cough, dyspnea and gastrointestinal disorders. However, their chest CT showed the typical signs of pneumonia as ground-glass opacity. All patients but two (TP57 and TP64) were treated with oxygen therapy at medium-low flows (FiO2 range 35–50%) for an average of 10 days of stay. Nobody passed away.

At the entrance, laboratory results showed a neutrophilia condition in one patient (TP57; 8.3 × 10³/mL, Table 4). Three individuals (TP61, TP64 and TP66) presented a relative lymphopenia (1.0 × 10³/mL ± 0.5, Table 4); three patients (TP57, TP61 and TP66) showed a slight rise in CRP levels (32.0 mg/L ± 26.9, Table 4) and an elevation of fibrinogen (723.0 mg/dl ± 146.5, Table 4). All but two (TP60 and TP64) presented high D-dimer values (mean 1681 ng/mL, Table 4).

3.5. ROC curve analysis

We conducted a ROC curve analysis to evaluate the capacity of overexpressed genes to identify COVID-19 patients from negative individuals. We tested a predictive model, combining the expression levels of ACE2 and DPP4 genes. Analysis of ROC curves revealed that the area under the ROC curve (AUC) was 0.75 with 95% confidence interval 0.63 to 0.87 (p-value < 0.001) (Figure 3).

3.6. Correlation analysis

No correlation was found among the expression data of the eight SARS-CoV-2 host invasion genes and the age of our 63 patients. We analyze, by Pearson correlation test, the relationship between the expression profile of the eight SARS-CoV-2 host invasion genes respectively in COVID-19 patients (n = 35) and negative subjects (n = 28) and we found a significant positive association among ACE2 mRNA and PCSK3, EMILIN1, MMRN1 and MMRN2 expression and between DPP4 mRNA and EMILIN2 expression only in COVID-19 patients (Figure 4). Moreover, we evaluate by Pearson correlation test, the relationship among the SARS-CoV-2 entry genes expression values and the clinical data of COVID-19 patients. A significant negative correlation was found among ACE2 expression level and lymphopenia in COVID-19 patients (Figure 5A) and also a significant negative association between DPP4 mRNA and CRP levels (Figure 5B).

Regression analyses performed in ACE2h-COVID-19 patients, indicate a significant positive correlation among ACE2 expression level and the number of neutrophils and LDH values (Figure 6). No correlation among DPP4 expression level and clinical data was found in DPP4h-COVID-19 patients.

4. Discussion

Several differences in clinical manifestations and complications of COVID-19 patients have been observed suggesting variability in the disease process [19]. Genetic variants in the host human genome, can in part explain the broad inter-individual variation of disease susceptibility and/or severity [6, 19, 20, 21]. Moreover, the expression levels of human genes encoding the main proteins involved in this mechanism could be critical for the susceptibility, symptoms and outcome of SARS-CoV-2 infection. Notably, elevated ACE2 expression promoted in vitro the susceptibility to SARS-CoV infection [22, 23]. Moreover, ACE2 is up-regulated in response to SARS-CoV-2 infection [24]. Interestingly, conditions like old age, obesity, chronic kidney disease (CKD) and chronic obstructive pulmonary disease (COPD) in which was reported DPP4 upregulation are associated with severe COVID-19 [25]. It was shown that DPP4 acted for Coronavirus (CoV) co-receptor, thus suggesting a potential similar mechanism of SARS-CoV-2 entry [26]. A recent study clearly reported a correlation between DPP4 and ACE2 expression, suggesting that both membrane proteins are relevant in the pathogenesis of virus entry [27].

The respiratory tract can be considered as a vulnerable target to SARS-CoV-2 infection [28, 29]. Therefore, we analyzed the expression level of eight genes involved in the virus entry in the upper respiratory tract, in particular nose and pharynx. We selected naso- and oropharyngeal swabs in the critical epidemic period 20th March–20th April 2020, during which a lot of seriously affected patients referred to Policlinico Tor Vergata of Rome. Our results indicate a significant nasal and oropharyngeal overexpression of ACE2 and DPP4 genes in COVID-19 patients and that this overexpression is not correlated with patients’ age. Moreover, to evaluate the ability of ACE2 and DPP4 levels to discriminate COVID-19 patients from negative subjects, we performed a ROC curve analysis. We evaluated the discriminating potential of the combined gene expression levels and we observed that they allow us to identify about 75% of COVID-19 patients (Figure 3). These results support the hypothesis that ACE2 and DPP4 expression level are altered in COVID-19 patients and that their combined evaluation could be a potential good discriminatory genomic biomarker. Interestingly, the expression level of ACE2 and DPP4 genes is significant higher respectively in a subgroup of seven (ACE2h-COVID-19) and six (DPP4h-COVID-19) COVID-19 patients (Figure 2). All seven ACE2h-COVID-19 patients except one presented dyspnea; two patients were intubated. CT showed abnormal images and the typical signs of pneumonia as ground-glass opacity. ACE2h-COVID-19 patients showed, at the entrance, a worse lymphopenia condition, a reduced cytokine pattern and lower levels of both fibrinogen and D-dimer (Table 2 vs Table 3). Accordingly, a recent meta-analysis demonstrated that admission lymphopenia and neutrophilia are associated with poor outcomes in patients with COVID-19 [30]. Lymphocytes express ACE2 on their surface and may represent a direct target of the virus. SARS-CoV-2 might directly infect them, resulting in lymphopenia that in turns might be related to lymphocytic dysfunction. Interestingly, our regression analysis demonstrated a significant negative correlation between ACE2 expression and the number of lymphocytes in COVID-19 patients (Figure 5A).

Based on these results we can hypothesize that subjects with higher expression level of ACE2 in nasopharyngeal and oropharyngeal cells are more vulnerable to develop more severe complications of COVID-19. The main function of ACE2 receptor is the downregulation of the renin-
angiotensin-system (RAS), balancing the overdrive of RAS mediated response and the renal, gastrointestinal absorption of amino acids. However, it also acts as a means of clathrin-mediated internalization of viruses such as SARS coronavirus [31]. By now, the functional role of the surface angiotensin-converting enzyme 2 (ACE2) as a receptor protein for viral entry is well known. This protein is widely found in different organs such as the lung, kidney, heart, and endothelial tissue. According to our results, other study report that in symptomatic patients affected by COVID-19 ACE2 expression is higher in alveolar epithelial cells [24, 29, 32]. Since an important therapeutic approach is to impair the fusion of SARS-CoV-2 with the type II pneumocytes ACE2 receptor, thus inhibiting and preventing any type of destruction of the type II pneumocytes, its overexpression in the nasopharyngeal and oropharyngeal swabs of severe COVID-19 patients when compared to negative subjects and to other positive patients, suggest that this approach may be useful also in the first step of infection, when ACE2 expression in nasopharyngeal and oropharyngeal tissue is high.

The six DPP4h-COVID-19 patients manifest a better clinical course than ACE2h-COVID-19 patients. In fact, their clinical symptom at onset was only fever, only four out six were treated with oxygen therapy at medium-low flows and no one of them died (Table 4). Laboratory analyses at the entrance were in the normal range for the most of them. To the best of our knowledge, none of these patients showed comorbidities associated to DPP4 over-expression as obesity and metabolic syndromes, thus suggesting a functional role of this receptor in the early phase of virus entry besides that of modifier gene of COVID-19 severity [33].

Dipeptidyl peptidase 4 (DPP4), also known as CD26, is a 110 kDa transmembrane glycoprotein expressed on the surface of a wide variety of epithelial cells and some lymphocytes. DPP4 is an important protease that is widely expressed on the surface of human cells and plays a key role in immune-regulation, inflammation, oxidative stress, cell adhesion, and apoptosis by targeting different substrates. DPP4 is also the main receptor for MERS-COV [34]. In docking analysis that examined the SARS-CoV-2 S protein and DDP4, a significant interaction between these proteins was found [15]. Interestingly, COVID-19 patients with COPD comorbidity show serious adverse outcomes and subjects with COPD express higher rates of DPP4 [35].

Recent studies have shown that the inhibition of DPP4 can exert antihypertensive effects by interfering with the function of the RAS system. Moreover, both ACE2 and DPP4 are proteins dysregulated in diabetes [36].

It might be possible that diabetic patients might be more affected by COVID-19 due to increased ACE2 and DPP4 levels that in turns mediate infection and contribute to a compromised vasculature [36].

Finally, our regression analysis found a positive association only in COVID-19 patients, among ACE2 expression level and PCSK3, EMILIN1, MMRN1, and MMRN2 ones and also between DPP4 and EMILIN2 mRNAs (Figure 4). This result suggests that a coordinated nasopharyngeal and
oropharyngeal expression level of these genes contribute to the virus entry and progression. Interestingly, regression analyses indicate also a significant negative correlation between ACE2 expression and the number of lymphocytes (Lym) ($R^2 = 0.190$, $p < 0.005$, Pearson $r = -0.436$; (B) DPP4 mRNA and CRP levels ($R^2 = 0.118$, $p < 0.05$, Pearson $r = -0.344$).

Figure 5. Correlation analysis of ACE2 and DPP4 m-RNA levels with clinical data in nasopharyngeal and oropharyngeal swabs of COVID-19 patients. Scatter plots depicting the relationship between (A) ACE2 mRNA and number of lymphocytes (Lym) ($R^2 = 0.190$, $p < 0.005$, Pearson $r = -0.436$; (B) DPP4 mRNA and CRP levels ($R^2 = 0.118$, $p < 0.05$, Pearson $r = -0.344$).

Figure 6. Correlation analysis of ACE2 m-RNA expressions with clinical data in nasopharyngeal and oropharyngeal swabs of ACE2h-COVID-19 patients. Scatter plots depicting the relationship between (A) ACE2 and number of neutrophils (neu) ($R^2 = 0.645$, $p < 0.05$, Pearson $r = -0.803$); (B) ACE2 and LDH levels ($R^2 = 0.578$, $p < 0.05$, Pearson $r = -0.760$).

Anyway, we only examined the expression of SARS-CoV-2 host invasion genes at the RNA level; consequently, we cannot explicitly conclude that these proteins are downregulated in COVID-19 patients.

The significant upregulation of ACE2 and DPP4 in COVID-19 patients suggest that their joint expression level might be a good discriminator of clinical outcome in COVID-19 patients and that both these receptors may play a complementary role in the virus entry and in disease progression. Moreover, our data suggest the therapeutic potential of known drugs targeted against ACE2 and DPP4 to treat or prevent COVID-19 symptoms in particular, derived vascular complications.

At the best of our knowledge, this is the first study that explores DPP4 expression in naso- and oropharyngeal swabs of COVID-19 patient demonstrating a significant upregulation of this receptor and suggesting its potential functional role in binding SARS-CoV-2 virus.

Declarations

Author contribution statement

F. Amati and G. Novelli: Conceived and designed the experiments; Wrote the paper.
C. Vancheri: Conceived and designed the experiments; Performed the experiments; Wrote the paper.
A. Latini: Performed the experiments; Analyzed and interpreted the data.
V. Colona, S. Loddo, A. Di Lorenzo, P. Rogliani and M. Andreoni: Analyzed and interpreted the data.
S. Grelli: Performed the experiments; Contributed reagents, materials, analysis tools or data.
M. D’Apice, E. Balestrieri, C. Passarelli and A. Minutolo: Contributed reagents, materials, analysis tools or data.

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