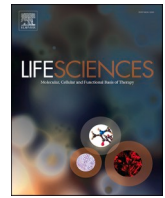




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## Dipeptidylpeptidase-4 levels and *DPP4* gene polymorphisms in patients with COVID-19. Association with disease and with severity

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### ABSTRACT

**Background:** The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the virus that causes de COVID-19 disease use as a principal receptor the angiotensin-converting enzyme-2 (ACE2). It has been suggested that dipeptidyl peptidase-4 (DPP4) can be another possible receptor for this virus. The present study aimed to establish if the DPP4 levels and DPP4 polymorphisms are associated with COVID-19 disease and its severity.

**Methods:** The study included 107 COVID-19 patients and 263 matched-healthy controls. Fifty patients required invasive mechanical ventilation. The DPP4 was quantified in serum using the Bioplex system. Based on the previous results and the functional prediction analysis, we select for the study 5 *DPP4* polymorphisms (rs12617336, rs12617656, rs1558957, rs3788979, and rs17574) and these were determined using the 5exonuclease TaqMan assays.

**Results:** Low levels of DPP4 were observed in COVID-19 patients (46.5 [33.1–57.7] ng/mL) when compared to healthy controls (125.3 [100.3–157.3] ng/mL) ( $P < 0.0001$ ). Also, patients that required mechanical ventilation showed lower DPP4 levels (42.8 [29.8–56.9] ng/mL) than those that did not need this procedure (49.2 [39.9–65.6] ng/mL) ( $P = 0.012$ ). DPP4 levels correlated negatively with age, fibrinogen, and platelet levels, and positively with albumin, alanine aminotransferase, and percentage of neutrophils. The *DPP4* rs3788979 polymorphism was associated with a high risk of COVID-19 disease and, the *TT* genotype carriers had the lowest DPP4 levels.

**Conclusions:** In summary, in the present study, an association of low levels of DPP4 with COVID-19 disease and severity was found. The association of the *DPP4* rs3788979 polymorphism with COVID-19 is also reported.

### 1. Introduction

Coronavirus disease 2019 (COVID-19) is a pandemic produced by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) that has produced 114,853,685 confirmed cases globally with 2554,694 deaths as of March 4, 2021 [1]. SARS-CoV-2 belongs to the Coronaviridae family and can infect humans and several animal species [2]. This virus has a high genetic similarity with SARS-like coronavirus (96%) and SARS-CoV (79.5%) [3]. It has been demonstrated that the virus uses the angiotensin converting enzyme-2 (ACE2) receptor and transmembrane

protease serine protease-2 (TMPRSS2) to enter the host cells [4]. Other proteases, such as cathepsins, furin, neutrophil elastase (ELANE), and probably TMPRSS11A [5–7] could be involved in this phenomenon. Another receptor for the corona-like viruses is the dipeptidyl peptidase 4 (DPP4), also known as CD26, which was reported to be the principal receptor for MERS-CoV cell entry [8]. MERS-CoV and SARS-CoV-2 possess structural similarities and are major causes of severe pneumonia in humans [9]. Indeed, using a modelled homo-trimer structure of COVID-19 spike glycoprotein that considered open (ligand-bound) and closed (ligand-free) conformation, Vankadari et al. established that the

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S1 domain of SARS-CoV-2 spike glycoprotein interacts human DPP4, which could be another receptor for the virus [10]. DPP4 is a protein that participates in various physiological processes and is present in two forms; it can be either anchored to the cell membrane or soluble, circulating in plasma [11]. Apart from its possible role in SARS-CoV-2 entry into the host cell, this protein has been significantly related to the presence of hypertension, obesity and insulin resistance [12–14]. Considering that DPP4 inhibitors increase half-life of glucagon-like peptide 1 (GLP-1), they have been used in the treatment of type 2 diabetes mellitus (T2DM) [15]. Considering that obesity and diabetes are important risk factors for SARS-CoV-2 infection and severity of COVID-19, the study of DPP4 protein in these patients can provide important information. A recent study in a very small group of individuals showed that patients hospitalized for COVID-19 have reduced levels of DPP4 compared to a control group [16]. This study also included a group of patients with sepsis, in whom no differences in DPP4 levels were found, suggesting that the decrease in this protein is conditioned by the SARS-CoV-2 infection. The DPP4-encoding gene is polymorphic; an association of the enzyme levels was found with not only some genotypes of these polymorphisms [17] but also some diseases, such as type 2 diabetes mellitus (T2DM) [17] and myocardial infarction [18]. Thus, the aim of the present study was to evaluate the DPP4 concentration in a group of patients with COVID-19 and establish if these levels are related with severity and some clinical findings. In addition, we evaluated the association of *DPP4* polymorphisms with SARS-CoV-2 infection, COVID-19 severity, and DPP4 levels.

## 2. Methods

### 2.1. Subjects

We enrolled 107 consecutive patients with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection confirmed by RT-PCR test in at least one biological sample. The patients were attended in the intensive care unit of our Institute from May 11 to July 31, 2020. Fifty patients required invasive mechanical ventilation. Demographic, clinical, and laboratory parameters of COVID-19 patients were obtained. All patients or their relatives signed the institutional consent letter.

To evaluate if DPP4 serum concentrations in COVID-19 patients were different from healthy controls, we used a case control study design. For each COVID-19 patient, we matched 3 controls by age, sex, and body mass index. Of the 107 COVID-19 patients, 83 patients were individually matched with 3 healthy controls, while 7 patients were matched with only 2 controls each. Therefore, in the present study, we examined circulating DPP4 serum concentrations in 90 COVID-19 patients and 263 healthy controls (172 men and 91 women). The healthy control individuals were selected among the participants of the Genetics of Atherosclerotic Disease (GEA) Mexican study control group [19]. The GEA control group included individuals without personal or family history of premature coronary disease. Anthropometric, biochemical, clinical, and demographic characteristics, as well as cardiovascular risk factors were determined in all controls as previously described [19–21]. Criteria definitions and methods have been reported previously [22–25]. Obesity [19,20], current smoking [20], hypertension [19,20], T2DM [19,20] definitions have already been published.

A serum DPP4 concentration  $\leq$  p25 (89.8 ng/mL for women and 108.1 ng/mL for men) was considered low. This cut-off point was obtained from a GEA study subsample of non-obese non-hypertense volunteers (124 men and 183 women) with normal values of fasting lipids and glucose.

### 2.2. Sample handling

The samples were obtained in the intensive care unit following the institutional security protocols. Competent trained personnel carried out the handling and processing of the patients' blood samples, which were

**Table 1**  
Characteristics of COVID-19 patients.

	All subjects (n = 107)
Male (%)	68
Age (years)	55.76 $\pm$ 13.59
Body mass index (Kg/m <sup>2</sup> )	29.08 $\pm$ 5.60
Comorbidities	
Hypertension (%)	45.8
Type 2 diabetes mellitus (%)	38.3
Obesity (%)	34.6
Dyslipidemia (%)	10.3
Ischemic heart disease (%)	8.4
Tabaquism (%)	7.5
Valvular disease (%)	4.7
Chronic kidney disease (%)	3.7
Hyperthyroidism (%)	3.7
Gout (%)	1.9
Systemic lupus erythematosus (%)	1.9
Antiphospholipid syndrome (%)	1.9
Pulmonary hypertension (%)	0.9
Initial symptoms	
Cough (%)	62.5
Fever (%)	61.7
Dyspnea (%)	56.1
Fatigue (%)	38.3
Myalgia (%)	38.3
Arthralgia (%)	33.6
Headache (%)	24.3
Odynophagia (%)	20.6
Diarrhea (%)	17.8
Diaphoresis (%)	12.1
Shaking chills (%)	11.2
Emesis (%)	9.3
Chest pain (%)	9.3
Anosmia (%)	7.5
Dysgeusia (%)	5.6
Abdominal pain (%)	5.6
Hyporexia (%)	4.7
Rhinorrhoea (%)	1.9

Data are shown as mean  $\pm$  standard deviation or percentage.

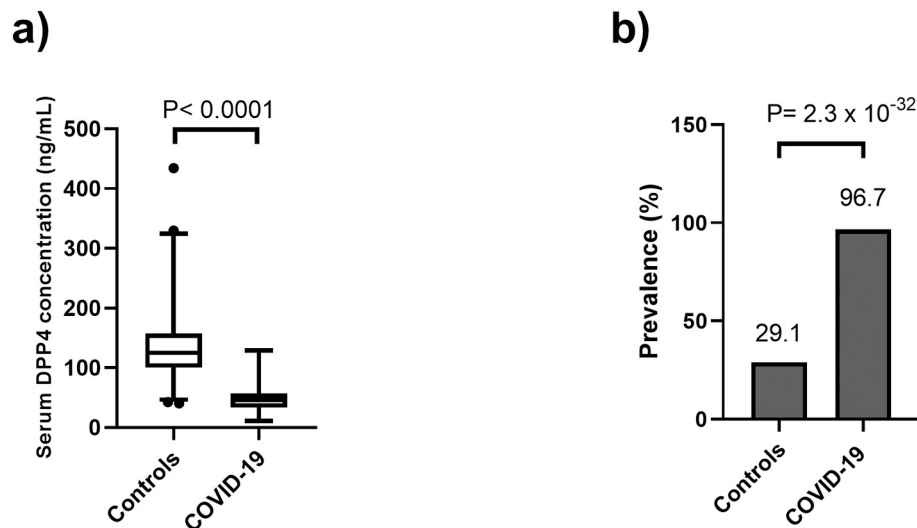
**Table 2**  
Demographic, clinical and biochemical parameters in COVID-19 patients and matched controls.

	All subjects (n = 353)	COVID-19 (n = 90)	Controls (n = 263)	P <sup>a</sup>
Male (%)	65.4	65.5	65.4	0.543
Age (years)	53.9 $\pm$ 10.9	54.5 $\pm$ 11.6	53.7 $\pm$ 10.6	0.509
Body mass index (Kg/m <sup>2</sup> )	28 [26–31]	28 [26–31]	28 [26–31]	0.340
Type 2 diabetes mellitus (%)	19.8	38.9	13.3	<0.001
Hypertension (%)	17.8	44.4	8.7	<0.001
Creatinine (mg/dL)	0.88 [0.71–1.06]	0.85 [0.59–1.35]	0.89 [0.74–1.04]	0.006
Alanine aminotranferase (U/L)	27 [20–44]	38 [21–62]	25 [19–37]	<0.001
Aspartate aminotranferase (U/L)	26 [21–35]	34 [22–55]	25 [21–31]	<0.001
Alkaline phosphatase (U/L)	80 [66–99]	88 [67–115]	79 [66–94]	0.010

Data are shown as mean  $\pm$  standard deviation, median [interquartile range] or percentage.

<sup>a</sup> Student's *t*-test, Mann Whitney's *U* test or Chi square test as appropriate.

later transported to the laboratory for processing. Samples were centrifuged, and the serum was separated in a class II biological safety cabinet. Personnel handling the samples used personal protective equipment that included disposable gloves, a lab coat, and a surgical



**Fig. 1.** DPP4 concentration and prevalence of low DPP4 in 90 COVID-19 patients matched with 263 healthy controls. a) serum DPP4 levels were significantly lower in COVID-19 patients (46.5 [33.1–57.7] ng/mL) compared with controls (125.3 [100.3–157.3] ng/mL,  $P < 0.0001$ ). b) Prevalence of low DPP4 concentrations in COVID-19 patients and healthy controls (96.7 vs 29.1,  $P = 2.3 \times 10^{-32}$ ).

mask. Samples were collected and processed in a laboratory that adheres to the guidelines established in the Official Mexican Standards NOR-007-SSA3-2011, NOM-087-SEMARNAT-SSA1-2002, NOM-010-SSA2-2010, NOM-006-SSA2-2013, and NMX-EC-15189 IMNC-2015.

### 2.3. Quantification of DPP4 serum concentration

Bioplex system (R&D Systems, Minneapolis, USA) was used to quantify DPP4 serum concentration, according to manufacturer's instructions. A Bio-Plex Manager software was used for the data analyses. Results are expressed in ng/mL.

### 2.4. Genetic analysis

For the genomic DNA isolation from whole blood (containing EDTA) of the COVID-19 patients, we used QIAamp DNA Blood Mini kit (QIAGEN, Hilden, Germany). The genomic DNA from the control individuals had been previously extracted and the aliquots were stored at  $-70^{\circ}\text{C}$ . We used the SNP Function Prediction (<http://snpinfo.niehs.nih.gov/snpinfo/snpfunc.html>), Human-transcriptome Database for Alternative Splicing (<http://www.h-invitational.jp/h-dbas/>), Splice Port: An Interactive Splice Site Analysis Tool (<http://spliceport.cbcb.umd.edu/SplicingAnalyser.html>), ESE finder (<http://rulai.cshl.edu/cgi-bin/tools/ESE3/esefinder.cgi>), HSF (<http://www.umd.be/HSF/>), and SNPs3D (<http://www.snps3d.org/>) bioinformatics tools to ascertain the possible functional effect of the *DPP4* polymorphisms. Based on the previous results and the functional prediction analysis, we select for the study 5 *DPP4* polymorphisms (rs12617336, rs12617656, rs1558957, rs3788979, and rs17574). Using 5' exonuclease TaqMan genotyping assays, these polymorphisms were genotyped on an ABI Prism 7900HT Fast Real-Time PCR system (Applied Biosystems, Foster City, CA).

### 2.5. Statistical analysis

Data are expressed as frequencies, median (interquartile range) or mean  $\pm$  standard deviation, as appropriate. For continuous variable comparisons, either Student's *t*-test or Mann-Whitney *U* test was used. The chi-squared test was employed for categorical variable comparisons. The frequencies of alleles and genotypes were determined by direct counting. We used the chi-squared test to determine the Hardy-

Weinberg's equilibrium. Haplotype analysis and linkage disequilibrium were performed with Haploview software (version 4.1, Broad Institute of Massachusetts Institute of Technology and Harvard University, Cambridge, MA, USA). DPP4 serum concentration comparisons were evaluated by the Mann-Whitney *U* or Kruskal-Wallis test, as appropriate. Using logistic regression analysis adjusted for age, sex, body mass index, hypertension and type 2 diabetes mellitus, we appraised the independence and significance of the following associations (a) COVID-19 infection and DPP4 serum levels, (b) COVID-19 infection and *DPP4* studied polymorphisms, and (c) DPP4 levels and the severity of COVID-19 infection (assessed by the need for mechanical invasive ventilation). The analysis of association of COVID-19 infection with each polymorphism under each inheritance model (additive, dominant, recessive, heterozygote, co-dominant1 and co-dominant2) was done individually. A *P* value  $< 0.05$  was considered significant. We used the SPSS software v15.0 (SPSS Chicago, IL) for all analyses.

## 3. Results

### 3.1. Clinical, biochemical, and demographic characteristics

One hundred-seven COVID-19 patients (68% male) were included in the study, with a median age of 55.76 years. Common symptoms were cough (62.5%), fever (61.7%), dyspnea (56.1%), fatigue (38.3%), and myalgia (38.3%). The most frequent comorbidities were hypertension in 45.8%, T2DM in 38.3%, and obesity in 34.6% (Table 1).

As mentioned above, of the 107 COVID-19 patients, 83 patients were individually matched with 3 healthy controls, and 7 patients with 2 controls each. Therefore, in the present study we examined the circulating DPP4 serum concentrations in 90 COVID-19 patients and 263 healthy controls; their clinical, demographic, and biochemical parameters are shown in Table 2. When compared to controls, COVID-19 patients showed a high prevalence of hypertension, T2DM, as well as high levels of alanine aminotransferase, aspartate aminotransferase, and alkaline phosphatase.

### 3.2. DPP4 levels in COVID-19 patients and healthy controls

COVID-19 patients showed significantly lower serum DPP4 levels (46.5 [33.1–57.7] ng/mL) when compared to healthy controls (125.3

**Table 3**  
Demographic, clinical and biochemical parameters of COVID-19 patients stratified by invasive mechanical ventilation.

	All patients (n = 107)	Invasive mechanical ventilation		P <sup>a</sup>
		Yes (n = 50)	No (n = 57)	
Age (years)	55.8 ± 13.6	58.4 ± 12.1	53.4 ± 14.4	0.055
Male (%)	55.1	82.0	56.1	0.004
Body mass index (Kg/m <sup>2</sup> )	27 [26–31]	28 [26–31]	27 [25–31]	0.672
Type 2 diabetes mellitus (%)	38.3	52.9	31.5	0.397
Hypertension (%)	45.8	55.9	41.1	0.093
Alanine aminotranferase (U/L)	49 ± 41	46 ± 35	51 ± 45	0.546
Aspartate aminotranferase (U/L)	47 ± 40	53 ± 41	42 ± 38	0.166
Alkaline phosphatase (U/L)	97 ± 51	100 ± 46	94 ± 55	0.527
Lactate deshydrogenase (U/L)	275 [224–383]	334 [260–436]	243 [194–296]	0.080
Total bilirubin (mg/dL)	0.60 [0.44–0.92]	0.55 [0.38–1.02]	0.69 [0.53–0.87]	0.552
Albumin (g/dL)	2.81 [2.53–3.22]	2.54 [2.36–2.79]	3.18 [2.84–3.36]	<0.001
C reactive protein (mg/L)	97.6 [27.9–204.1]	135.5 [55.1–231.3]	73.9 [21.4–153.0]	0.013
D-Dimer (µg/mL)	0.51 [0.24–0.90]	0.75 [0.49–1.79]	0.29 [0.17–0.64]	<0.001
Fibrinogen (g/mL)	5.01 ± 1.33 [355–998]	5.18 ± 1.30 [404–1142]	4.85 ± 1.34 [336–853]	0.201
Ferritin (g/mL)	634 [355–998]	771 [404–1142]	556 [336–853]	0.041
Lymphocytes %	11.8 [6.0–16.3]	8.4 [3.3–13.3]	14.6 [9.5–14.6]	<0.001
Neutrophils %	43.4 ± 40.5	39.1 ± 43.8	47.1 ± 37.3	0.313
Leucocytes × 10 <sup>3</sup> /µL	7.5 [5.5–11.4]	9.4 [6.4–14.4]	6.0 [4.7–8.8]	<0.001
Platelets × 10 <sup>3</sup> /µL	288.0 ± 125.6	291.1 ± 119.3	285.3 ± 131.8	0.813
International normalized ratio	1.21 ± 0.36	1.19 ± 0.22	1.22 ± 0.45	0.752
Mortality (%) <sup>b</sup>	27.5	47.9	9.3	<0.001

Data are shown as mean ± standard deviation, median [interquartile range] or percentage.

<sup>a</sup> Student's t-test, Mann Whitney's U test or Chi square test as appropriate.

<sup>b</sup> This analysis was made in 102 patients.

[100.3–157.3] ng/mL) ( $P < 0.0001$ ) (Fig. 1a). In the same way, the prevalence of low levels of DPP4 was higher in COVID-19 patients than in controls (96.7 vs 29.1,  $P = 2.3 \times 10^{-32}$ ) (Fig. 1b). We used logistic regression analyses adjusted for age, sex, body mass index, hypertension and type 2 diabetes mellitus to evaluate the independence and significance of the association between COVID-19 and DPP4 serum levels. We found that a low DPP4 concentration ( $<p25$ ) was associated with a significantly higher risk of COVID-19 (OR [95%CI] = 70 [20–230],  $P = 1.69 \times 10^{-11}$ ) (Data not shown).

### 3.3. DPP4 levels in COVID-19 patients with and without mechanical ventilation

DPP4 levels were compared in patients who required invasive mechanical ventilation (IMV) and those who did not. The clinical and demographic characteristics of these groups are shown in Table 3. In this analysis, we included 107 COVID-19 patients. Individuals who underwent IMV (82.0% male) had high levels of C-reactive protein, d-dimers, ferritin, and leucocytes when compared to non-seriously ill patients who

did not require ventilation. The group of patients who received IMV showed low levels of DPP4 (42.8 [29.8–56.9] ng/mL) compared to individuals without ventilation (49.2 [39.9–65.6] ng/mL) ( $P = 0.012$ ) (Fig. 2a). A marginal difference in DPP4 levels was observed between patients who recovered from the disease (48.9 [35.8–63.1] ng/mL) and those who died (42.5 [30.3–55.6] ng/mL) ( $P = 0.070$ ) (Fig. 2b). The analysis of mortality was made in 102 patients, because 5 patients were transferred to another hospital and we did not know the outcome. Under different models adjusted for confounding variables (age, sex, BMI, T2DM, and hypertension), high DPP4 levels were associated with low risk of requiring IMV (Table 4).

In three COVID-19 patients from the total of 107, DPP4 levels were measured several days after the diagnosis and their admission to the intensive care unit. As can be seen in Fig. 3, in the two COVID-19 patients who recovered without IMV, serum DPP4 levels increased, whereas they decreased in one mechanically ventilated patient.

### 3.4. Correlation of DPP4 levels with clinical characteristics in COVID-19 patients

DPP4 levels correlated negatively with age ( $r = -0.192$ ,  $P = 0.048$ ), fibrinogen ( $r = -0.214$ ,  $P = 0.027$ ) and platelet levels ( $r = -0.268$ ,  $P = 0.005$ ), and positively with albumin ( $r = 0.416$ ,  $P = 0.001$ ), alanine aminotranferase ( $r = 0.333$ ,  $P = 0.001$ ), and percentage of neutrophils ( $r = 0.241$ ,  $P = 0.012$ ) (Fig. 4).

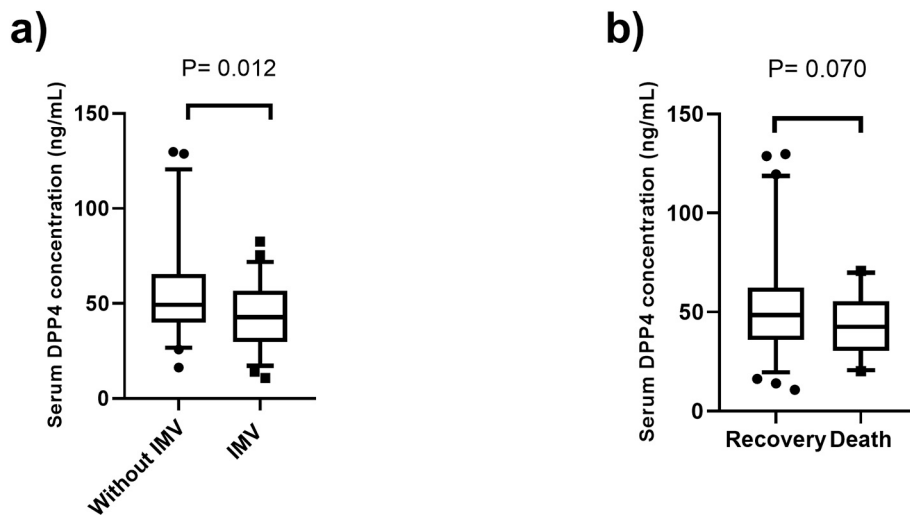
### 3.5. Association of DPP4 polymorphisms with COVID-19

Of the 5 polymorphisms studied, rs3788979 showed a different distribution of its genotypes in COVID-19 patients and healthy controls. Under different models adjusted for age, sex, BMI, T2DM, and hypertension, rs3788979 was associated with a high risk of COVID-19 (OR = 2.36, 95% CI: 1.60–3.48,  $P_{\text{additive}} = 1.4 \times 10^{-5}$ ; OR = 2.54, 95% CI: 1.45–4.45,  $P_{\text{dominant}} = 0.001$ ; OR = 4.28, 95% CI: 2.12–8.62,  $P_{\text{recessive}} = 4.7 \times 10^{-5}$ ; OR = 1.88, 95% CI: 1.03–3.41,  $P_{\text{codominant 1}} = 0.04$ ; OR = 6.07, 95% CI: 2.76–13.34,  $P_{\text{codominant 2}} = 7.0 \times 10^{-6}$ ) (Fig. 4). On the other hand, in the total population (patients and controls), different DPP4 levels were observed in the rs3788979 genotypes; of note, the lowest levels were found in the TT genotype carriers ( $P = 0.003$ ) (Fig. 5).

## 4. Discussion

In a cohort of COVID-19 patients and controls, we found significantly lower levels of DPP4 in COVID-19 patients. Enzyme levels were also low in patients receiving IMV; by the same token, a low risk of requiring IMV was associated with a high DPP4 concentration. High enzyme levels correlated negatively with age, fibrinogen, and platelets, and positively with albumin, ALT, and percentage of neutrophils. The rs3788979 DPP4 polymorphism was associated with a high risk of COVID-19.

DPP4 is an enzyme that has been associated with the presence of hypertension, insulin resistance, and T2DM [13,14,17,26]. At the same time, this protein has been reported to be the receptor for corona-like viruses, such as MERS [27]. With a bioinformatics analysis, it has been recently established that the spike protein of the SARS-CoV-2 interacts with DPP4 [10], suggesting a possible role of this protein in the infection and severity of the disease. In a small number of COVID-19 patients, Schlicht et al. [16] reported low levels of DPP4 as compared to healthy controls; this result had been previously reported in MERS-CoV infected subjects [28]. DPP4 is a ubiquitous serine peptidase that is expressed in endothelial, bronchiolar epithelial, alveolar epithelial [29], and blood cells (particularly lymphocytes) [30]. This protein is found in two forms: one bound to the cell membrane and another one dissolved in plasma. Concerning the SARS infection, DPP4 could have several functions; one could be its role in the entry of the virus to the



**Fig. 2.** DPP4 concentration in COVID-19 patients stratified: a) without invasive mechanical ventilation (IMV) (n = 57, 49.2 [39.9–65.6] ng/mL) and with IMV (n = 50, 42.8 [29.8–56.9] ng/mL) and b) those who recovered (n = 74, 48.9 [35.8–63.1] ng/mL) and who died (n = 28, 42.5, [30.3–55.6] ng/mL). The analysis of mortality was made in 102 patients, because 5 patients were transferred to another hospital and we did not know the outcome.

**Table 4**  
Association between serum DPP4 concentration and invasive mechanical ventilation in COVID-19 patients.

	Odds ratio [95% confidence interval]	P
Unadjusted	0.970 [0.949–0.992]	0.008
Model 1	0.971 [0.949–0.994]	0.013
Model 2	0.973 [0.951–0.996]	0.022
Model 3	0.974 [0.951–0.997]	0.025
Model 4	0.972 [0.949–0.996]	0.021
Model 5	0.974 [0.950–0.998]	0.031

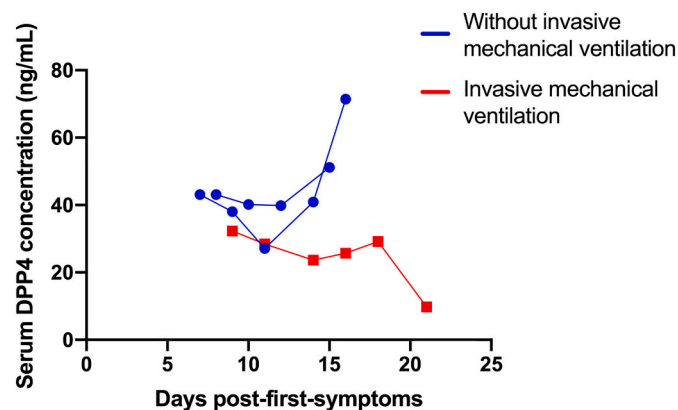
Model 1: adjusted for age > 60 years.

Model 2: Model 1 + adjusted for sex.

Model 3: Model 2 + adjusted for body mass index.

Model 4: Model 3 + adjusted for type 2 diabetes mellitus.

Model 5: Model 4 + hypertension.



**Fig. 3.** DPP4 concentrations in three COVID-19 patients measured several days after the diagnosis and their admission to the intensive care unit. Two COVID-19 patients who recovered without invasive mechanical ventilation and one mechanically ventilated patient are showed.

host cell. Another function could be the systemic effect that it may have, considering that it is produced by the adipose tissue as a proinflammatory adipokine with an important effect on the T cell activation, cell adhesion, and apoptosis [31]. Its relationship with the inflammatory process and insulin resistance is relevant since T2DM and obesity are

major risk factors for a severe course of a COVID-19 disease [32]. Recently Solerte et al., showed that the DPP4 inhibitor Sitagliptin is beneficial in COVID-19 because this type of DPP4 inhibitor induces a compensatory up-regulation of DPP4 expression and release [33]. This result agrees with our findings. High circulating levels of DPP4 have been reported in individuals with obesity in several populations with an important correlation with insulin resistance [26,34]. However, the relationship of the plasma DPP4 activity with obesity and T2DM is contradictory; increased and decreased activity levels in patients with T2DM and obesity have been reported [35–38]. Similarly, low levels of DPP4 have been reported in some conditions, such as rheumatoid arthritis [39], pregnancy [40], and hypoalbuminoproteinemia [personal communication]. These findings appear to be contrary to those expected. However, epigenetic modifications and changes at DNA level are part of the complex mechanisms that modulate the production of several biomolecules, including DPP4.

It is difficult to establish whether low DPP4 levels in patients are a cause or consequence of SARS-CoV-2 infection, however, the fact that uninfected control individuals have higher levels of DPP4 suggests that variation at low of this enzyme could be a consequence of the infection. In this context, low serum levels of DPP4 in COVID-19 patients could be due to several mechanisms; one would involve the enzyme as a receptor for the virus, which could modify the structure of the protein at the cell membrane, preventing its proteolytic cleavage. Hence, individuals infected with SARS would consequently have a lower DPP4 serum level, which is demonstrated in our study. Another mechanism affecting the soluble enzyme concentration could involve alterations at the intracellular level caused by the virus, which could prevent an adequate assembly of DPP4. This effect could decrease the amount of biomolecule in the cell membrane and, consequently, in the serum of the patients. As mentioned, DPP4 is expressed in several blood cells, but predominantly in T lymphocytes [30] and specifically in CD4 T cells [41]. Measurement of DPP4 in lymphocyte culture has suggested that these cells are an important source of soluble DPP4 [42]. As in other coronavirus infections (SARS and MERS), the individuals infected with SARS-CoV-2 present with a marked lymphopenia [43–45]; this disorder is more severe in those individuals who do not survive the disease, while in surviving patients, T lymphocyte levels are gradually restored [46]. Considering these facts, the decreased number of lymphocytes (the primary cell source of soluble DPP4) could explain why low levels of DPP4 in serum are present in COVID-19 patients. Another possible function of DPP4 as it relates to COVID-19 is that it could act as a ‘decoy

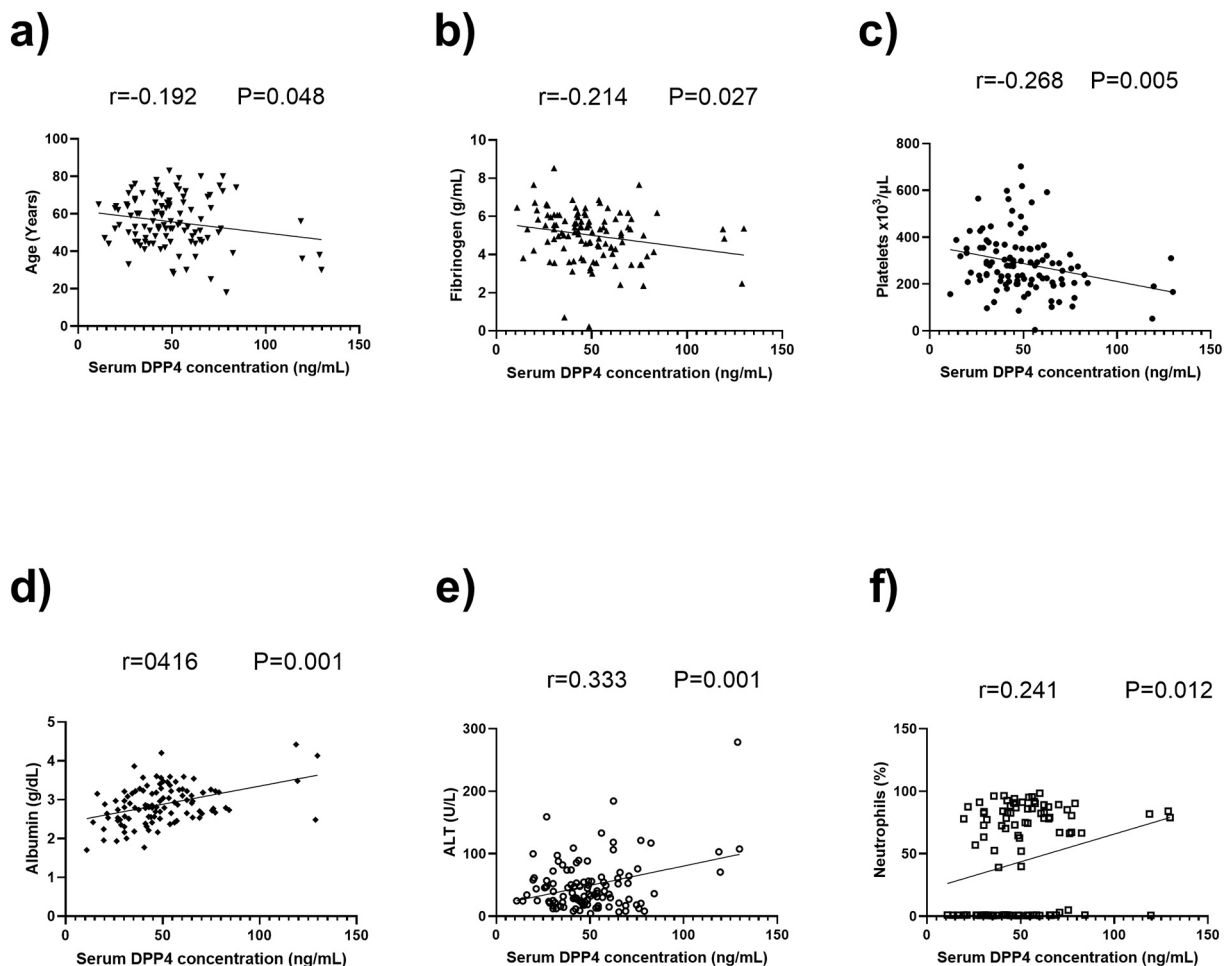


Fig. 4. Pearson correlations between DPP4 levels and quantitative variables in COVID-19 patients:  $r$  and  $P$  values are shown.

receptor' for the SARS-CoV-2. In this case, viral particles bound to circulating DPP4 could be partially or totally preventing from entering cells. Thus, high circulating DPP4 levels would be expected to reduce the spread of the virus within the host, while low levels would be related to a greater entry of the virus into the cells and consequently a greater degree of infection. We suggest that the latter is what would be happening in the patients analyzed in our study.

It has been suggested that the variability in SARS-CoV-2 infection prevalence and COVID-19 mortality in different populations depend largely on individual variations in genes that encode proteins relevant in viral pathogenicity mechanisms. Bioinformatics, case-control and genome-wide association (GWA) studies have shown polymorphisms located in several genes as possible susceptibility markers for COVID-19 infection and severity in several populations [47–51]. Located in the 2p24.3 region, the gene that encodes DPP4 is polymorphic and some of its polymorphisms have been associated not only with the risk of developing some diseases, such as myocardial infarction [18], rheumatoid arthritis [52], and T2DM [17], but also with variations in levels of DPP4 [17] and apolipoprotein B [53]. In our study, an association of the *DPP4* rs3788979 polymorphism with risk of COVID-19 was established. We also found that the lowest levels of this enzyme were present in *TT* genotype carriers; this polymorphism was previously associated with risk of myocardial infarction in patients with atherosclerosis [18]. In agreement with our results, Aghili et al. reported that individuals with the rs3788979 *CT/TT* genotypes presented with low levels of DPP4.

Recently, a GWA study of 2244 patients with COVID-19 from the UK detected 3 polymorphisms associated with severe COVID-19, one of

them located in the dipeptidyl peptidase 9 (*DPP9*) gene (rs2109069) [54]. *DPP9* and *DPP4* belong to the S9B subfamily of peptidases with high structural resemblance [55]. Indeed, *DPP9* participates in the immune and inflammatory processes. This enzyme is expressed in leukocytes [56], activated lymphocytes [57], lymphocytes infiltrating inflamed lungs [58], and bronchi after induction of experimental asthma [59].

A strength of our study is that patients were matched for age and sex with the control group. An important limitation of this work is that we did not measure the activity of the soluble or the membrane-bound DPP4. This is important because the two DPP4 forms could have a different effect in the SARS-CoV-2 infection and then in the COVID-19 disease. Another source of uncertainty is that the DPP4 variation during the course of the disease was only tracked in three individuals, and yet there was agreement in their levels. In the two individuals who did not require IMV, the enzyme concentration increased, while it diminished in the one who did receive mechanical ventilation.

In summary, the presence and severity of COVID-19 is associated with lower levels of DPP4, which could be a possible marker for disease monitoring during its course. Individuals with rs3788979 *TT* genotype might produce low levels of DPP4 and, as a result, be more susceptible to infection and progression of the disease.

#### Declaration of competing interest

The authors declare that they have no conflict of interest.

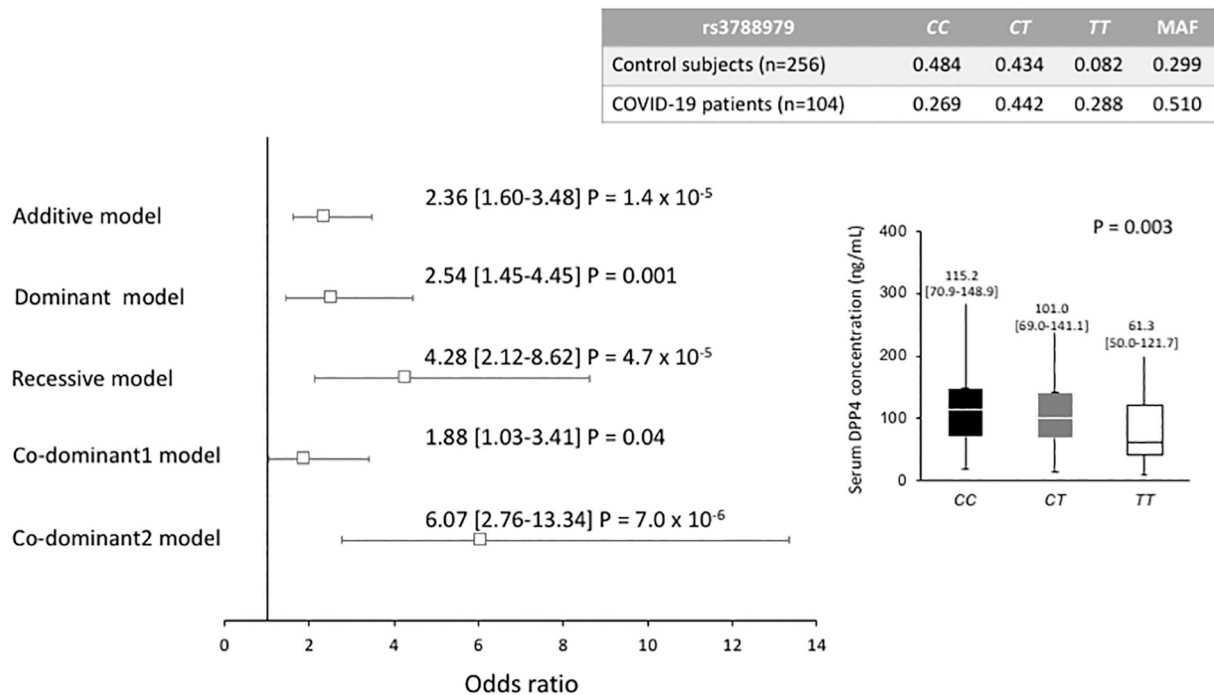


Fig. 5. Association between *DPP4* rs3788979 gene polymorphism and COVID-19 infection and *DPP4* serum concentration stratified by rs3788979 genotypes. Each model was adjusted for age, sex, body mass index, type 2 diabetes mellitus and hypertension. MAF: Minor allele frequency.

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