



Early View

Research letter

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Effect of ACE1 polymorphism rs1799752 on protein levels of ACE2, the SARS-CoV-2 entry receptor, in alveolar lung epithelium

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Take-Home message: Increased protein levels of ACE2 in alveolar epithelium of subjects who are homozygous for the *ACE1* insertion of rs1799752 might facilitate host cell entry of SARS-CoV-2 and explain the higher prevalence of COVID-19 in certain regions.

To the editor,

Coronavirus Disease 19 (COVID-19), caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is currently invoking a pandemic with a huge medical and financial impact. One of the striking features of this pandemic is the considerable variation in disease presentation and severity amongst patients, ethnic groups, and countries. This variation can be partially explained by differences in population density, demographic factors (age, sex) and comorbidities (e.g. hypertension, obesity and diabetes mellitus). Also genetic factors likely contribute to SARS-CoV-2 infection risk and/or COVID-19 development.

SARS-CoV-2 host cell attachment, the first step in the host cell entry process, is predominantly facilitated by the Angiotensin-Converting Enzyme 2 (ACE2) receptor (1, 2). ACE2 is part of the ACE2/angiotensin-(1-7)/Mas axis and counteracts the effects of its homologue ACE1, which is involved in the ACE1/angiotensin II/Angiotensin I Receptor (AT1R) axis of the renin angiotensin aldosterone system (RAAS). An ACE1/ACE2 imbalance has been suggested to play an important role in SARS-CoV-2 infectivity and COVID-19 progression (3).

The ACE1 gene is characterized by a genetic deletion/insertion (D/I) of an alu repeat in intron 16 and this polymorphism (rs1799752) shows an important geographical variation (4). Strikingly, 60% of ACE1 levels in blood seem to be determined by this D/I polymorphism (5, 6). This profound influence can be explained by the presence of two different promoters and alternative splicing resulting in two isoforms of the gene (7). Since ACE1 and ACE2 levels are strongly regulated by common genetic variants in their genes (3), ACE2 levels may also be influenced by this polymorphism.

Recently, we showed that COVID-19 incidence was inversely correlated to the presence of the ACE1 D-allele frequency (8). Also, a significant correlation between COVID-19 related mortality and the prevalence of the D-allele was observed. Furthermore, other genes associated to RAAS (SLC6A20 and

ABO) have been picked up with genome-wide significance for severe COVID-19 with respiratory failure (9). Interestingly, the ABO-locus modulates a quantitative variation in ACE1 levels (10). Furthermore, the link between severe COVID-19 and hypertension, diabetes, and cardiovascular disease raises the hypothesis of genetic predisposition of RAAS genes and severe COVID-19.

We determined ACE2 protein expression in lung tissue of different patient groups (patient characteristics: **Figure 1A**) (11). Briefly, ACE2 protein expression was visualized by immunohistochemistry (IHC) on formalin-fixed paraffin-embedded lung tissue blocks using anti-ACE2 antibody (Abcam: ab15348; isotype: Rabbit IgG, R&D systems, AB-150-C) and quantitative measurements of the ACE2-positive signal in alveolar tissue were performed using Axiovision software (Zeiss, Oberkochen, Germany). Representative images of the ACE2 IHC staining (including isotype control) are shown in **Figure 1B**. We now postulate that increased ACE2 levels might be – at least partially – attributed to genetic variance in the ACE1 gene. Therefore, we determined the prevalence of the three genotypes (DD, DI, II) of the D/I polymorphism in our patient cohort (n = 67). Genotypes of rs1799752 in *ACE1* were determined using Taqman® SNP Genotyping assays (Thermo scientific, C_6053859A_10 and C_6053859B_20) according to the manufacturer's instructions (**Figure 1C**). The present study was approved by the medical ethical committee of the Ghent University Hospital (BC-08811).

We found a significant increase of ACE2 protein levels in alveolar lung epithelium when patients were homozygous for the insertion (II) (**Figure 1D**). Importantly, this correlation remained significant, even after adjusting for age, sex, BMI, diabetes, smoking, and COPD (**Figure 1E**). For this reason, we propose that the D/I polymorphism in *ACE1* contributes to the variation in alveolar protein expression of ACE2, the SARS-CoV-2 entry receptor, and thus also to the infectivity and pathogenicity of the virus.

It should be noted that the sample size of this study is limited to perform genetic research resulting in an underrepresentation of the *ACE1* II genotype. Another limitation of the present study is the lack of proof for a direct link between *ACE1* polymorphism and ACE2 protein expression. Further research in larger and more geographically distributed cohorts, as well as experiments concerning the link between the polymorphism and ACE2 expression (e.g. gain/loss of function studies) are needed to confirm our findings.

In conclusion, we suggest a genetic deletion/insertion polymorphism in *ACE1* to associate with ACE2 protein levels in lung tissue thereby potentially affecting infectivity by SARS-CoV-2. Due to the geographical variance in the *ACE1* D/I genotype (3, 4), this might contribute to the varying prevalence, morbidity, and mortality due to COVID-19.

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Figure 1. ACE2 protein levels in alveolar lung epithelium according to allele of rs1799752

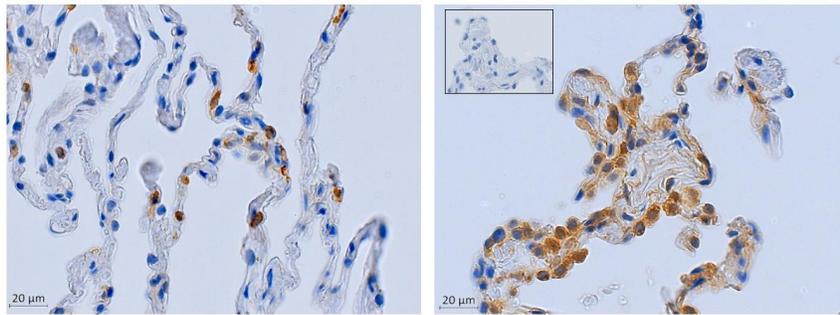
A) Table with patient characteristics. No significant differences between groups were found with a Mann-Whitney U test for continuous outcomes or a Fisher's exact test for binomial outcomes. B) Representative images of a ACE2 low (left) and mid-to-high range (right) score IHC staining, showing positive signal in alveolar tissue, at a 400x magnification. The small inlay is representative of the negative isotype control staining. C) Graph depicting mean values of PCR fluorescent signal of 2 replicates per sample in the VIC (533-580 nm, assay C_60538594B_20) and FAM (465-510 nm, assay C_60538594A_10) channels. D) Bar plot depicting median values of ACE2 expression in alveolar epithelium, normalized for the total alveolar tissue of subjects with DD (n=19), DI (n=42), and II (n=6) *ACE1* genetic variants. Error bars represent 2.5 – 97.5 percentiles. * $p < 0.05$ according to the unpaired t-test on the natural logarithm transformed ACE2 expression values. E) Forest plots depicting regression coefficients from linear regression analyses with determinant median ln values of ACE2 expression (n = 67) and adjusted for age, female sex, BMI, DI (compared to DD), II (compared to DD), diabetes, smoking (compared to never smoking without COPD), and COPD (compared to never smoking without COPD). P values for regression for age, female sex, BMI, DI, II, diabetes, smoking, and COPD are 0.030, 0.296, 0.035, 0.149, 0.002, 0.002, 0.089 and 0.011 respectively.

BMI = body mass index. COPD = Chronic Obstructive Pulmonary Disease. st.dev. = standard deviation. D = deletion. I = insertion.

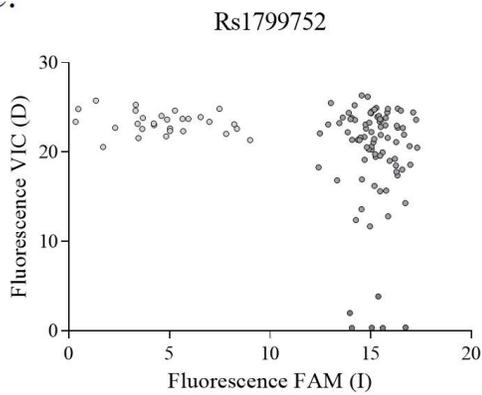
A.

	DD	DI	II
Subjects (%)	N = 19 (13.4)	N = 42 (62.7)	N = 6 (8.9)
Age (mean, st.dev.)	62.7 (7.9)	64.0 (10.3)	64.8 (7.5)
Female sex (%)	8 (42.1)	17 (40.5)	4 (66.7)
BMI (mean, st.dev.)	26.6 (7.1)	26.2 (5.1)	25.9 (6.3)
Smoking			
Never (%)	4 (21.1)	12 (28.6)	2 (33.3)
Ex (%)	5 (26.3)	9 (21.4)	1 (16.7)
Current (%)	10 (52.6)	21 (50.0)	3 (50.0)
Pack-years (mean, st.dev.)	31.7 (20.1)	26.6 (22.1)	35.5 (39.9)
Comorbidities			
Diabetes (%)	3 (15.8)	4 (9.5)	0 (0)
COPD (%)	9 (47.4)	15 (35.7)	3 (50.0)
Hypertension (%)	8 (42.1)	23 (54.8)	3 (50.0)

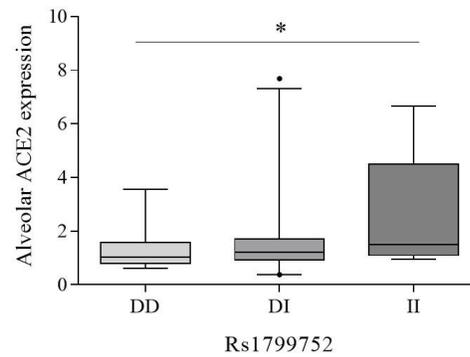
B.



C.



D.



E.

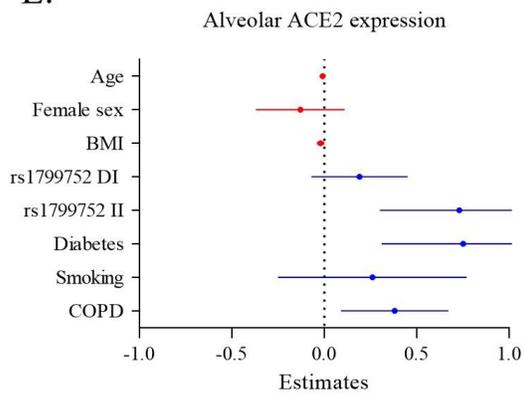


Figure 1.