ORIGINAL RESEARCH LETTER

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

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 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 characterised 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 promotors 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 the lung tissue of different patient groups (patient characteristics are shown in figure 1a) [11]. Briefly, ACE2 protein expression was visualised 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

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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 #SARSCoV2 and explain the higher prevalence of #COVID19 in certain regions https://bit.ly/3k6aAE8

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a)

	DD	DI	II
Subjects	19 (13.4)	42 (62.7)	6 (8.9)
Age years	62.7±7.9	64.0±10.3	64.8±7.5
Female sex	8 (42.1)	17 (40.5)	4 (66.7)
BMI kg·m^{-2}	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)
Exposure pack-years	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)

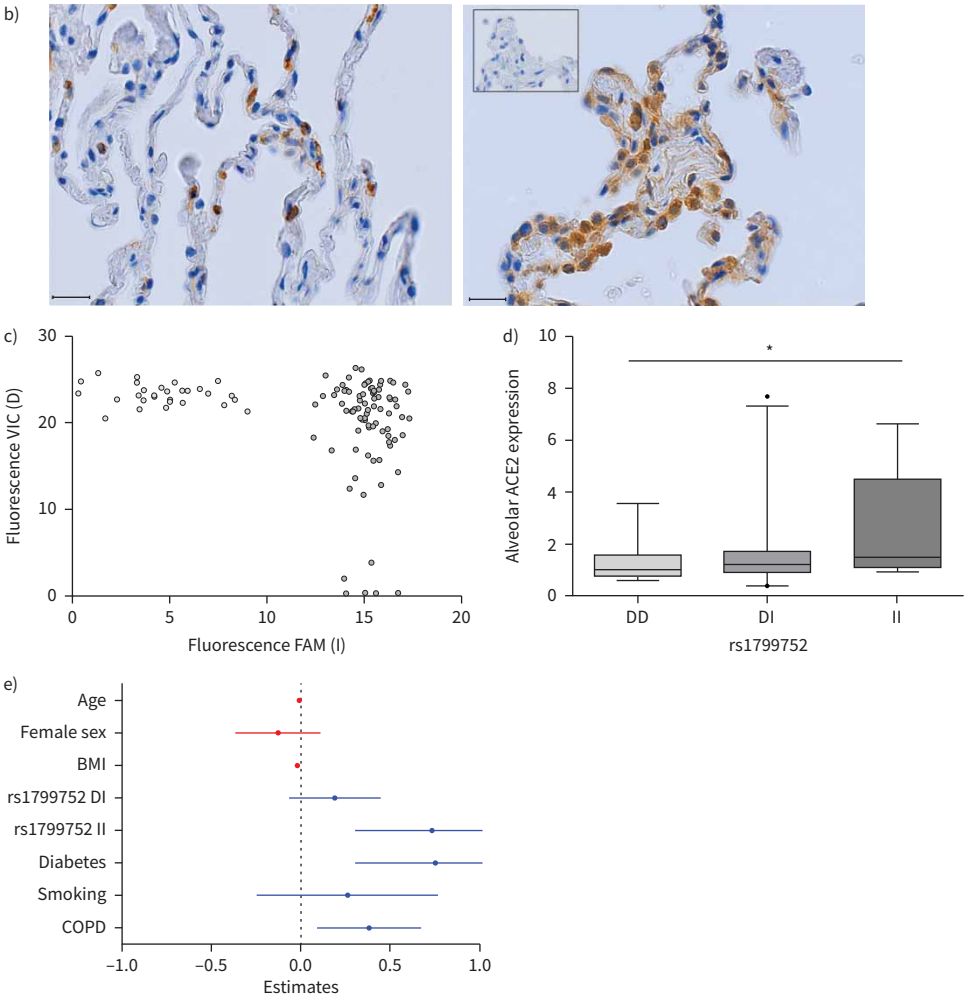

FIGURE 1 Angiotensin-converting enzyme 2 (ACE2) protein levels in alveolar lung epithelium according to allele of rs1799752. a) Patient characteristics grouped according to the three genotypes (DD, DI, II) of the deletion/insertion (D/I) polymorphism. No significant differences between groups were found using Mann–Whitney U-test for continuous outcomes or Fisher's exact test for binomial outcomes. Data are presented as n (%) or mean±SD. b) Representative images of a ACE2 low (left) and mid-to-high range (right) score immunohistochemistry staining, showing positive signal in alveolar tissue, at a 400× magnification. The small inlay is representative of the negative isotype control staining. Scale bars=20 µm. 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, normalised for the total alveolar tissue of subjects with DD (n=19), DI (n=42), and II (n=6) *ACE1* genetic variants. Error bars represent 2.5th–97.5th 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 with DD), II (compared with DD), diabetes, smoking (compared with never-smoking without COPD), and COPD (compared with 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.

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 in 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, body mass index, 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* associates 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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