ACE2 polymorphism and susceptibility for SARS-CoV-2 infection and severity of COVID-19

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Objectives **The RNA virus severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is responsible for coronavirus disease 2019 (COVID-19). Cell entry is mediated by the human angiotensin-converting enzyme II (ACE2). *ACE2* and its close homolog angiotensin-converting enzyme I (*ACE*) are currently discussed candidate genes, in which single-nucleotide polymorphisms (SNPs) could alter binding or entry of SARS-CoV-2 and enhance tissue damage in the lung or other organs. This could increase the susceptibility for SARS-CoV-2 infection and the severity of COVID-19.**

Patients and methods **We performed genotyping of SNPs in the genes *ACE2* and *ACE* in 297 SARS-CoV-2-positive and 253 SARS-CoV-2-negative tested patients. We analyzed the association of the SNPs with susceptibility for SARS-CoV-2 infection and the severity of COVID-19.**

Results **SARS-CoV-2-positive and SARS-CoV-2-negative patients did not differ regarding demographics and clinical characteristics. For *ACE2* rs2285666, the GG genotype or G-allele was significantly associated with an almost two-fold increased SARS-CoV-2 infection risk and a three-fold increased risk to develop serious disease or COVID-19 fatality. In contrast, the *ACE* polymorphism was not related to infection risk or severity of disease. In a multivariable analysis, the *ACE2* rs2285666 G-allele remained as an independent risk factor for serious disease besides the known risk factors male gender and cardiovascular disease.**

Conclusions **In summary, our report appears to be the first showing that a common *ACE2* polymorphism impacts the risk for SARS-CoV-2 infection and the course of COVID-19 independently from previously described risk factors. *Pharmacogenetics and Genomics* 31: 165–171**

Pharmacogenetics and Genomics 2021, **31**:165–171

Keywords: ACE2, coronavirus disease 2019, rs2285666, severe acute respiratory syndrome coronavirus 2, single-nucleotide polymorphism

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Received 27 January 2021 **Accepted** 16 March 2021

Introduction

The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), a member of the family of *Coronaviridae*, is responsible for coronavirus disease 2019 (COVID-19). The most common symptoms are fever, cough, and dyspnea [1]. Despite intensive research, it remains to be unraveled why some individuals get infected while others remain uninfected under similar conditions (e.g. medical history or exposure to SARS-CoV-2). In addition, it is unclear why the course of the disease differs drastically between different subjects with similar preconditions ranging from mild symptoms to death with an overall fatality rate of around 2% (25 January 2021) and an in-hospital mortality ranging from 28 to 72% [2,3]. The

Supplemental Digital Content is available for this article. Direct URL citations appear in the printed text and are provided in the HTML and PDF versions of this article on the journal's website, www.pharmacogeneticsandgenomics.com.

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main risk factors discussed for infection and severity are older age, male gender, obesity, and a variety of disorders such as hypertension, diabetes, and coronary heart disease, to name but a few [1,2,4].

Virus entry into host cells depends on binding of the viral spike (S) protein to membrane-bound angiotensin-converting enzyme II (ACE2) [5], which is localized on, for example, oral mucosa, respiratory tract, and heart cells [6,7]. Its main physiological function is the degradation of angiotensin II (Ang II) to angiotensin 1–7 (Ang 1–7) which activates the vasorelaxant mas-related G-protein coupled receptor [8]. Many authors have postulated that single-nucleotide polymorphisms (SNPs) in the gene *ACE2* (Xp22.2) could affect its expression and also the binding affinity of SARS-CoV-2, thereby influencing the susceptibility for SARS-CoV-2 infection and severity of COVID-19 [9–12]. The best characterized SNP in *ACE2* is a splice region variant (rs2285666, G>A, Intron 3/4). Several studies have shown associations with hypertension, coronary heart disease, and diabetes combined with cerebral stroke [13–15]. Interestingly, the A-allele is associated with increased serum levels of ACE2 in healthy controls, subjects with diabetes, and patients with diabetes along with cerebral stroke [13]. Although it is unknown whether the increased serum ACE2 levels associated with the A-allele actually also reflect increased membrane-bound ACE2 in, for example, the lung or other tissues, this polymorphism appears to be an attractive candidate associated with an altered susceptibility for infection or course of COVID-19 given the fact that ACE2 functions as a cellular 'receptor' for virus entry.

Another frequently discussed gene is *ACE* (17q23.3) coding for angiotensin-converting enzyme (ACE), usually cleaving angiotensin I (Ang I) to Ang II, which activates the angiotensin II receptor type 1 (AT1R) mediating vasoconstrictive, pro-inflammatory, and -fibrotic effects [8]. A deletion/insertion (D/I) of an Alu repeat (rs1799752, Intron 16) in the gene *ACE* may also play a role in COVID-19 [11]. *ACE* rs1799752 D-allele carriers show significantly higher ACE serum levels than carriers of the I-variant [16]. While the D-allele is associated with an increased risk for, for example, myocardial infarction or left ventricular hypertrophy [17,18], it is also a risk factor for the development and progression of acute respiratory distress syndrome (ARDS) and SARS [19–22]. Recently, Yamamoto *et al.* demonstrated in an in-silico study, that *ACE* rs1799752 II genotype is negatively correlated with number of SARS-CoV-2 cases and deaths in East Asia [23]. In a first case-control study, encompassing 204 SARS-CoV-positive patients, the *ACE* rs1799752 DD genotype was associated with a higher risk for severe COVID-19 [24].

Thus, those variants in the genes *ACE2* and *ACE* are good candidates in genetic association studies trying to learn more about the genetic causes of susceptibility for infection with SARS-CoV-2 and the severity of COVID-19.

Methods

Study participants and recruitment

The study was conducted following the approval of the ethics committees of the Medical Faculties of the University of Duisburg-Essen (20-9230-BO), Ruhr-University Bochum (20-6886), and RWTH Aachen University (206/09) and in cooperation with the West German Biobank (WBE; 20-WBE-088) and the RWTH centralized Biomaterial Bank (RWTH cBMB; 31-2020). Written informed consent was obtained from the study patients.

Enrolment from the respective hospitals or centers (Essen, Aachen, Herne, or Mettmann) started on 11 March 2020 and ended on 30 September 2020. Patients were initially recruited upon presentation with COVID-19 typical symptoms, that is, fever, cough, and dyspnea, or who were admitted to the hospital/center with already confirmed SARS-CoV-2 infection. We included 297 SARS-CoV-2-positive patients. Follow-up was completed on 31 October 2020, at which time all patients either were discharged from the hospitals as 'cured' or had a fatal outcome of the disease. We also studied 253 SARS-CoV-2-negative patients, who presented with COVID-19 typical symptoms but were tested SARS-CoV-2-negative by real-time reverse-transcription-PCR (RT-PCR). Those patients were hospitalized at the University Hospital Essen or treated as outpatients, due to other medical conditions. SARS-CoV-2 positivity or negativity was determined as described below. Samples from 200 anonymous blood donors collected before the COVID-19 pandemic served as a reference cohort.

SARS-CoV-2 *in vitro* diagnostic test

Nasopharyngeal swabs were collected and SARS-CoV-2 RNA positivity was determined by qualitative RT-PCR with *in vitro* diagnostic kits according to manufacturers' instructions. Patients were classified as SARS-CoV-2-positive with at least one positive RT-PCR test result and as SARS-CoV-2-negative with exclusively negative RT-PCR test results and no COVID-'typical' or COVID-'possible' chest computed tomography (CT) result (see below).

Chest computed tomography

A diagnostic chest CT scan was performed with default settings for thoracic routine when patients presented with clinical signs indicating an infection of the lower respiratory tract defined as dyspnea, tachypnea (breathing rate > 25), cough, a decreased oxygen saturation (<92%), or crackles [25]. All images were performed in one of the three CT systems (Somatoform Force, Somatom Definition AS+, Somatom Definition Flash; Siemens Healthineers, Erlangen, Germany). All CT examinations were re-evaluated especially for this study by a board-certified senior radiologist blinded for SARS-CoV-2 RT-PCR test results. Diagnosis of SARS-CoV-2 pneumonia was established as follows: multifocal bilateral distribution

of ground-glass opacity with/without consolidations in lung regions close to visceral pleural surfaces (including fissures) or signs of ARDS [1,26]. SARS-CoV-2 RT-PCR-negative patients, who presented any of these features ('typical') or atypical CT results, which could be related to COVID-19 pneumonia ('possible') were excluded from the study ($N = 69$) since sensitivity of chest CT was found to be superior to RT-PCR [27]. The results of chest CT scans from SARS-CoV-2-positive patients served as internal quality control ($N = 182$).

Demographics, clinical characteristics, and outcome of the patients

Demographics, clinical characteristics, and outcomes were obtained from the electronic patient records. For each patient's age, sex, medical history, medication of angiotensin-converting-enzyme inhibitors (ACEi), and angiotensin II receptor blockers (ARBs), initial symptoms (fever, cough, and dyspnea [1]), and outcome were collected. Medical history was classified based on the European Centre of Disease Prevention and Control (ECDC) [28] as cardiovascular system (e.g. myocardial infarction, coronary heart disease, but not arterial hypertension), arterial hypertension, or diabetes. Clinical outcome was defined as follows according to the criteria of the ECDC – 'mild': outpatients; 'hospitalized': inpatients; 'severe': hospitalized patients admitted to an ICU or became dependent on mechanical ventilation, or both; 'fatal' all cases of COVID-19-related deaths during the hospital stay. In contrast to the ECDC classification, where patients are counted up to three times, every patient only counted once according to the worst clinical outcome observed during the hospital stay in our study. The main group 'moderate' consisted of the subgroups 'mild' and 'hospitalized', whereas the main group 'serious' consisted of the subgroups 'severe' and 'fatal'.

Genotyping of ACE2 and ACE

PCR was performed with 100 ng genomic DNA and 30 µL *Taq* DNA-Polymerase 2x Master Mix Red (Ampliqon, Odense, Denmark) with the following conditions: initial denaturation 95°C for 5 min; 38 cycles with denaturation 95°C for 30 s, annealing temperature (Tm) °C for 30 s and elongation 72°C for 30 s each; final elongation 72°C for 10 min. Oligonucleotide sequences and Tm for the respective polymorphisms can be found in Supplemental Digital Content 1, *http://links.lww.com/FPC/B393*. Genotyping was performed for *ACE2* rs2285666 and *ACE* rs1799752 by Pyrosequencing according to the manufacturers' instructions (Qiagen, Hilden, Germany). For *ACE2* coding region polymorphisms (amino acids 1-62, MAF ≥ 0.005, Supplemental Digital Content 2, Supplemental Digital Content 1, *http://links.lww.com/FPC/B393*) only previously identified SNPs with potential functional impact on SARS-CoV-2 binding were analyzed by Sanger sequencing [9,10].

Statistical analyses

Correlations of demographics and clinical characteristics with SARS-CoV-2 infection risk were analyzed by Fisher's exact test. Differences between age groups in SARS-CoV-2-positive and SARS-CoV-2-negative patients were estimated by Mann–Whitney test.

Hardy–Weinberg equilibrium (HWE) for *ACE* was calculated using Pearson's χ^2 goodness of fit test and samples were considered as deviant from HWE at a significance level of $P < 0.05$. HWE for X-chromosomal *ACE2* was tested with the R-script 'HWadmiX' [29].

For genetic association, we calculated odds ratio (OR) and 95% confidence interval (CI) by Fisher's exact test using Baptista-Pike method for OR, respectively. *P*-values are reported two-sided and values of < 0.05 were considered significant.

Multivariable analysis was performed to estimate independence of the variables age, sex, medical history, medication, and *ACE* rs1799752 or *ACE2* rs2285666 genotypes by logistic regression (likelihood ratio test, backward).

Results

Demographics, clinical characteristics, and outcome of the patients

From March 11, 2020, to October 31, 2020, we enrolled and studied 297 SARS-CoV-2-positive and 253 SARS-CoV-2-negative patients to determine the associations of SNPs in the genes *ACE2* and *ACE* with susceptibility for SARS-CoV-2 infection and severity of COVID-19.

SARS-CoV-2-positive and SARS-CoV-2-negative patients had similar demographics and clinical characteristics (Table 1). Considering cardiovascular diseases except for hypertension, we observed a significantly higher prevalence in the SARS-CoV-2-negative patients (48.2% vs. 36.1%, $P = 0.01$). There were no differences regarding ACEi or ARBs medication in both groups. Cough and dyspnea were the most frequent initial symptoms in the SARS-CoV-2-positive group ($P < 0.0001$ and $P = 0.03$, respectively). We found significantly more SARS-CoV-2-positive patients than SARS-CoV-2-negative patients in the 'serious' group ('severe' plus 'fatal'; $P = 0.001$). Comparing the 'moderate' and the 'serious' groups, we found a significant association for the severity of COVID-19 with age ($P = 0.02$), male sex (OR: 2.25, 95% CI, 1.32–3.84, $P = 0.003$), and cardiovascular disease (OR: 1.84, 95% CI, 1.10–3.08, $P = 0.02$) (Table 2).

Association of ACE2 and ACE genotypes with susceptibility for SARS-CoV-2 infection and severity of COVID-19

For *ACE2* rs2285666, we found 77.4% GG, 13.5% GA, and 9.1% AA genotype carriers in SARS-CoV-2-positive patients, with a minor allele frequency (MAF) of 0.16 for the A-allele. Genotypes were compatible with HWE

Table 1 Demographics, clinical characteristics, and outcome of the patients

Characteristics		All patients (*N*=550)	SARS-CoV-2-positive (*N*=297)	SARS-CoV-2-negative (*N*=253)	*P*
Median age (range) – yrs.		62.0 (18–99)	60.0 (18–99)	63.0 (22–97)	0.17
Male sex – no. (%)		323 (58.7)	176 (59.3)	147 (58.1)	0.79
Medical history – no. (%)					
Cardiovascular system[a]		225 (41.7)	104 (36.1)	121 (48.2)	0.01
Arterial hypertension		266 (49.3)	133 (46.2)	133 (52.8)	0.14
Diabetes		138 (25.6)	66 (22.9)	72 (28.7)	0.14
Medication – no. (%)[b]					
ACEi		129 (23.9)	61 (21.2)	68 (27.1)	0.13
ARBs		85 (15.8)	44 (15.3)	41 (16.3)	0.81
Initial symptoms – no. (%)					
Fever		224 (46.7)	107 (46.1)	117 (47.2)	0.85
Cough		164 (34.2)	103 (44.4)	61 (24.6)	<0.0001
Dyspnea		185 (38.5)	101 (43.5)	84 (33.9)	0.03
Outcome – no. (%)[c]					
Moderate –	Mild	67 (12.1)	30 (10.1)	37 (14.6)	0.12
	Hospitalized	347 (63.1)	177 (59.6)	170 (67.2)	0.07
Serious –	Severe	66 (12.0)	44 (14.8)	22 (8.7)	0.03
	Fatal	70 (12.7)	46 (15.5)	24 (9.5)	0.04

ECDC, European Centre of Disease Prevention and Control; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.
[a]Cardiovascular system: e.g. myocardial infarction, coronary heart disease but not arterial hypertension.
[b]Only angiotensin-converting-enzyme inhibitors (ACEi) and angiotensin II receptor blockers (ARBs) were analyzed.
[c]Classification according to the COVID-19 surveillance report of the ECDC: category 'mild' is a case that has not been reported as hospitalized or dead. Whereas a 'severe' case has been admitted to intensive care and/or required respiratory support.

(*P*=0.53). The genotypes in SARS-CoV-2-negative patients were distributed as follows: 70.4% GG, 13.8% GA, and 15.8% AA (Table 3). The A-allele had a MAF of 0.23. There was no deviation from HWE (*P*=0.65). Interestingly, we noticed that the genotype distribution of our reference cohort fitted in the middle between SARS-CoV-2 infected and non-infected patients (MAF = 0.18; HWE *P*=0.97). We observed 1.91-fold (95% CI, 1.13–3.24, *P*=0.02) and 1.88-fold (95% CI, 1.12–3.16; *P*=0.02) increased susceptibility for SARS-CoV-2 infection for *ACE2* rs2285666 GG genotypes or G-allele carriers, respectively (Table 4).

We noted an almost linear decrease of the MAF of the A-allele from the 'mild' (23%), over the 'hospitalized' (19%) to the 'severe' (9%) and the 'fatal' (7%) group in SARS-CoV-2-positive patients. We found a three-fold increased risk for *ACE2* rs2285666 GG genotype carriers to develop a 'serious', rather than a 'moderate' course of infection (OR: 3.04, 95% CI, 1.47–6.27, *P*=0.002). We observed an almost three-fold increased fatality risk for *ACE2* rs2285666 GG genotype carriers comparing patients from the 'fatal' group (*N*=46, 15.5%) to all other SARS-CoV-2-positive patients (OR: 2.69, 95% CI, 1.02–7.11, *P*=0.05). Remarkably, only one of the patients in the 'fatal' group carried the *ACE2* AA genotype. Although limited by a small number of patients with 'mild' course of disease (*N*=30), we found a trend for association with a two-fold increased risk for hospitalization (groups: 'hospitalized', 'severe' and 'fatal') for *ACE2* rs2285666 GG genotype carriers (OR: 2.18, 95% CI, 0.98–4.85, *P*=0.06).

Table 2 Association of demographics and clinical characteristics of SARS-CoV-2-positive patients with COVID-19 severity

Characteristics	Moderate[a] (*N*=207)	Serious[a] (*N*=90)	OR (95% CI)	*P*
Median age (range) – yrs.	58.0 (18–94)	64.0 (26–99)	NA	0.02
Male sex – no. (%)	111 (53.6)	65 (72.2)	2.25 (1.32–3.84)	0.003
Medical history – no. (%)				
Cardiovascular system[b]	63 (31.7)	41 (46.1)	1.84 (1.10–3.08)	0.02
Arterial hypertension	89 (44.7)	44 (49.4)	1.21 (0.73–1.99)	0.52
Diabetes	43 (21.6)	23 (25.8)	1.26 (0.71–2.26)	0.45
Medication – no. (%)[c]				
ACEi	37 (18.6)	24 (27.0)	1.62 (0.90–2.91)	0.12
ARBs	31 (15.6)	13 (14.6)	0.93 (0.46–1.87)	0.86

OR, odds ratio; CI, confidence interval; *P*, *P* value as calculated for estimation of significant differences ($P<0.05$) of the patients` age, sex, medical history or medication, and COVID-19 severity; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.
[a]The group 'moderate' consisted of the subgroups 'mild' and 'hospitalized', whereas the group 'serious' consisted of the subgroups 'severe' and 'fatal'.
[b]Cardiovascular system: e.g. myocardial infarction, coronary heart disease but not arterial hypertension.
[c]Only angiotensin-converting-enzyme inhibitors (ACEi) and angiotensin II receptor blockers (ARBs) were analyzed.

A coding region variant in the gene *ACE2* was only observed in three SARS-CoV-2-negative and in one SARS-CoV-2-positive patient (rs4646116, c.77A>G, p.K26R, MAF = 0.006).

In SARS-CoV-2-positive and SARS-CoV-2-negative patients, the *ACE* rs1799752 genotypes were equally distributed (Table 3). There was no deviation from HWE in SARS-CoV-2 negative patients (*P*=0.32), but genotypes in the SARS-CoV-2-positives were deviant from HWE (*P*=0.02). No significant association for the D/I variant with susceptibility for SARS-CoV-2 infection or course of COVID-19 was observed (Table 4).

A multivariable logistic regression showed that the *ACE2* rs2285666 G-allele (OR: 3.75, 95% CI, 1.23–11.47, *P*=0.02), male sex (OR: 2.75, 95% CI, 1.57–4.80, *P*=0.00002), and cardiovascular disease (OR: 1.79, 95% CI, 1.06–3.05, *P*=0.03) remained as independent significant predictors for 'serious' course of COVID-19.

Discussion

Influence of demographics, medical history, and medication on SARS-CoV-2 infection risk and COVID-19 severity

We found a similar prevalence of distribution of medical history and in-hospital mortality as observed in a recent large-scale observational study with 10.021 German hospitalized COVID-19 patients [2], suggesting our study cohort may be representative for hospitalized patients. We could not find associations between sex, age, medical history, or ACEi/ARBs medication and SARS-CoV-2 infection risk, which might be related to our highly selected cohort with SARS-CoV-2-negative patients, who presented at our hospital for other severe medical conditions than COVID-19. This may as well explain the higher prevalence of patients with cardiovascular diseases in the SARS-CoV-2-negative group. Considering

Table 3 Genotype distributions of *ACE2* and *ACE* single-nucleotide polymorphisms in SARS-CoV-2-positive, SARS-CoV-2-negative patients and reference group

	SARS-CoV-2-positive					SARS-CoV-2-negative	Reference group
		Moderate		Serious			
Group[a] Subgroup	All (*N*=297) no. (%)	Mild (*N*=30) no. (%)	Hospitalized (*N*=177) no. (%)	Severe (*N*=44) no. (%)	Fatal (*N*=46) no. (%)	All (*N*=253) no. (%)	All (*N*=200) no. (%)
ACE2 rs2285666 GG	230 (77.4)	19 (63.3)	131 (74.0)	39 (88.6)	41 (89.1)	178 (70.4)	150 (75.0)
ACE2 rs2285666 GA	40 (13.5)	8 (26.7)	26 (14.7)	2 (4.6)	4 (8.7)	35 (13.8)	29 (14.5)
ACE2 rs2285666 AA	27 (9.1)	3 (10.0)	20 (11.3)	3 (6.8)	1 (2.2)	40 (15.8)	21 (10.5)
minor allele frequency	0.16	0.23	0.19	0.09	0.07	0.23	0.18
ACE rs1799752 DD	105 (35.4)	9 (30.0)	65 (36.7)	21 (47.7)	10 (21.7)	77 (30.4)	55 (27.5)
ACE rs1799752 DI	126 (42.4)	12(40.0)	74 (41.8)	17 (38.6)	23 (50.0)	118 (46.6)	95 (47.5)
ACE rs1799752 II	66 (22.2)	9 (30.0)	38 (21.5)	6 (13.6)	13 (28.3)	58 (22.9)	50 (25.0)
minor allele frequency	0.43	0.50	0.42	0.33	0.53	0.46	0.49

ECDC, European Centre of Disease Prevention and Control; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.
[a]For clinical outcome we defined four subgroups ('mild', 'hospitalized', 'severe', and 'fatal') to classify our patient cohort according to the criteria of the ECDC. Patients in the 'mild' group consisted of outpatients. The 'hospitalized' group encompassed patients, who stayed in the hospital as inpatients. The 'severe' group comprised of hospitalized patients admitted to an ICU and/or became dependent on mechanical ventilation. The 'fatal' group counted all cases of death during the hospital stay. The group 'moderate' consisted of the subgroups 'mild' and 'hospitalized', whereas the group 'serious' consisted of the subgroups 'severe' and 'fatal'.

Table 4 Association of *ACE2* rs228566 and *ACE* rs1799752 with SARS-CoV-2 infection risk

ACE2, rs2285666 G>A	G vs. A	GG + GA vs. AA	GG vs. AA
OR (95% CI), *P* value	1.57 (1.03–2.41), *P*=0.04	1.88 (1.12–3.16), *P*=0.02	1.91 (1.13–3.24), *P*=0.02
ACE, rs1799752 D/I	D vs. I	DD + DI vs. II	DD vs. II
OR (95% CI), *P* value	1.12 (0.80–1.57), *P*=0.65	1.04 (0.70–1.55), *P*=0.92	1.20 (0.76–1.90), *P*=0.48

CI, confidence interval; D, deletion; I, insertion; OR, odds ratio; *P*, *P* value as calculated for estimation of significant differences ($P<0.05$) of the patients' genotypes and SARS-CoV-2 infection risk; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

only the SARS-CoV-2-positive patients, we could find a significantly higher risk for COVID-19 severity in male patients or those with cardiovascular diseases confirming the data from recent observational studies [4,30].

ACE2 rs2285666 GG genotype or G-allele and risk for SARS-CoV-2 infection

Currently, a number of studies analyze the impact of genetic host factors on SARS-CoV-2 infection risk or COVID-19 severity. Notably, one such genetic factor was unraveled in a genome-wide association study to be the blood group A, with an approximately 1.45-fold increased infection risk compared to other blood groups [31]. Other authors – mainly due to hypothetical reasons – have postulated that polymorphisms in the genes encoding for important components of the renin-angiotensin system, that is, *ACE* or *ACE2* could play a role in infection risk. In a pilot study, Novelli *et al.* sequenced the whole exomes of 131 hospitalized COVID-19 patients and 1000 individuals of an Italian control group and evaluated the association of *ACE2* variants with COVID-19 [32]. Although *ACE2* rs2285666 was observed in this study (MAF = 0.18) the allele frequency did not differ between SARS-CoV-2-positives and the control group. The comparison to a control group with uncertain SARS-CoV-2 status may be a drawback of this study. In fact, we could show the MAF of *ACE2* rs2285666 from healthy blood donors collected before the COVID-19 pandemic fitted in-between infected and non-infected patients. In the comparison of SARS-CoV-2-positive to SARS-CoV-2-negative patients; however, we determined an almost two-fold increased risk for SARS-CoV-2 infection in *ACE2* rs2285666 GG carriers or G-allele carriers compared to AA genotypes. The mechanism behind this finding remains elusive or even counterintuitive. If the notion is correct that the A-allele is associated with increased ACE2 serum levels or even cellular ACE2 expression [13] one would expect – if ACE2 acts as the 'receptor' for SARS-CoV-2 entry – an increased risk for A-allele carriers. Presently, we have no explanation for our findings. It should; however, be noted that our cohort is not representative of the general population since it consisted of a highly selected cohort of both severely sick SARS-CoV-2-positive and -negative patients entering the emergency wards of highly specialized University hospitals. In addition, while in the SARS-CoV-2-positive group the infection was clearly present as determined by RT-PCR, SARS-CoV-2-negativity may be regarded as less reliable. We cannot exclude the viral load in these subjects was too low for the detection or whether they might have had different exposure to SARS-CoV-2.

Coding region variants in the gene *ACE2* seem to play a negligible role, although they might be functional [33] since those variants were too rare to contribute to infection risk. Furthermore, *ACE* genotype distribution was not significantly different between SARS-CoV-2-positive and -negative individuals.

ACE and ACE2 polymorphisms and severity of COVID-19

We found no effect of *ACE* rs1799752 D/I polymorphism on the natural course of COVID-19. This stays in contrast to a recent Spanish study by Gomez *et al.* who reported an increased risk for an unfavorable COVID-19 outcome in *ACE* DD genotype carriers [24]. The reason for this discrepancy is unclear. Moreover, since genotyping errors can be excluded we have no explanation for the observation that *ACE* genotypes slightly violated HWE in the SARS-CoV-2-positive but not in the SARS-CoV-2-negative group or in healthy blood donors. This could hypothetically be attributed to an unrecognized selection bias in the first-mentioned group.

On the other hand, and again in contrast to the findings of Gomez e*t al.*, we found a significant association of the *ACE2* rs2285666 G-allele with a three-fold increased risk for an unfavorable COVID-19 course. Interestingly, the frequency of the A-allele decreased almost linearly from 23% in 'mild' disease to only 7% in the 'fatal' group. Despite the limited sample size, we found a significant association between COVID-19 fatality risk and GG genotype as well.

Multivariable logistic regression analysis confirmed the G-allele as an independent risk factor for a 'serious' course of COVID-19 besides the known risk factors male gender and cardiovascular disease.

It has been postulated that an ACE/ACE2 imbalance may play a central role in COVID-19 [34,35]. In this scenario, SARS-CoV-2 infection decreases the activity of ACE2 which typically counteracts the pro-inflammatory and -fibrotic effects of Ang II by converting it to Ang 1–7. If one accepts this theory, our findings suggesting a 'protective' role of the *ACE2* rs2285666 A-allele would make sense: If A-allele carriers actually produce more ACE2 than those with GG genotypes, A-allele carriers could be protected at least partially against the ACE/ACE2 imbalance and potentially better deal with the detrimental effects of increased Ang II levels, which could cause severe lung and heart injury [35,36].

A word of caution is nevertheless necessary: our Caucasian study population was limited and highly selected. Therefore, our findings cannot be transferred to the general population or populations with different ethnicities. Before universally being acceptable, our findings and hypothesis require a confirmation from independent investigations.

In summary, our report appears to be the first showing that a common *ACE2* polymorphism impacts the risk for SARS-CoV-2 infection and the course of COVID-19 independently from previously described risk factors.

Acknowledgements

We kindly thank Iris Manthey, Grit Müller and Stephanie Büscher for technical support (Institute of Pharmacogenetics). We thank Dr. Katharina Jockers, Tanja Lehmann and Heike Loewendick for their support regarding patient samples from the WBE (University Hospital Essen), Jennifer Wipperfürth for her help with RWTH cBMB samples (University Hospital Aachen) and Dr. Lothar Volbracht, Dr. Marc Wichert, Martina Wachsmann and Petra Kasper for their support with the samples from the central laboratory (University Hospital Essen).

Conflicts of interest

This work was supported by a grant (to Dr. Birte Möhlendick) from the Stiftung Universitätsmedizin Essen of the Medical Faculty Essen. The funder of the study had no role in study design, data collection, data analysis, data interpretation, writing of the report, or in the decision of submitting the article for publication. For the remaining authors, there are no conflicts of interest.

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