Faustine I, Marteka D, Amelia L, Kurniawati S, Malik A, Andrajati R, Wanandi SI, Supriyanto E. Distribution of ACE gene I/D genotypes and clinical characteristics of patients with hypertension and COVID-19 in Indonesia. Pharmacy Practice 2024 Apr-Jun;22(2):2914.
https://doi.org/10.18549/PharmPract.2024.2.2914

Original Research

Distribution of ACE gene I/D genotypes and clinical characteristics of patients with hypertension and COVID-19 in Indonesia

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Received (first version): 01-Jul-2023 Accepted: 13-Sep-2023 Published online: 02-May-2024

Abstract

Background: Suppression of the renin-angiotensin system by SARS-CoV-2 binding changes the balance between ACE and ACE2, which affects blood pressure regulation. The ACE gene polymorphisms in intron 16 are associated with susceptibility to SARS-CoV-2 infection in patients with hypertension. **Objective:** This study analyzed the ACE gene polymorphism distribution and determined the probability of infection and severity of hypertension in COVID-19 patients. **Methods:** One hundred and six adult subjects were involved in this cross-sectional study, comprising 95 COVID-19 subjects and 91 non-COVID-19 subjects from two parts of Indonesia in 2021, i.e. Palu City, Central Celebes, and Lahat District, South Sumatra, DNAs extracted from whole blood were analyzed for I/D polymorphisms by Polymerase Chain Reaction (PCR) method. **Results:** Distribution of ACE genotypes were found as follows; II (53%), ID (38%), and DD (9%). The percentage of hypertension and the severity of COVID-19 in the Palu population were higher than those in Lahat District, i.e., 44% vs. 14% and 80% vs. 46%, respectively. Although there was no significant association between the I/D genotypes and susceptibility or severity of COVID-19 ($p > 0.05$), it appeared that subjects with hypertension and dyspnea symptoms were five times more susceptible to a moderate-severe symptom that required hospitalization and was associated with a fivefold increase in the risk of dyspnea symptoms that required hospitalization. However, comorbid hypertension was associated with moderate to severe COVID-19 ($p=0.007$). **Conclusion:** It can be assumed that in our studied population, ACE gene I/D polymorphisms and hypertension are not associated with susceptibility to SARS-CoV-2 infection, but the presence of comorbid hypertension is a risk factor for more severe COVID-19.

Keywords: hypertension; COVID-19; ACE gene; insertion-deletion; SARS-CoV-2

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INTRODUCTION

The SARS-CoV-2 virus that causes coronavirus disease 2019 (COVID-19), which emerged in early 2020, was recognized by the World Health Organization (WHO) as a global health threat in March 2020, and COVID-19 was declared a global pandemic with a high mortality rate. Since the outbreak of COVID-19, 634 million cases have been recorded globally. Indonesia reported ±1% of cases, with a case fatality rate of 2.4%, which is higher than the global case fatality rate of 1.2% (until November 21, 2022).[1]

COVID-19 is classified as mild, moderate, or severe, depending on the severity of the illness. Patients may develop mild symptoms such as fever and may self-isolate at home or require intubation and mechanical ventilation in the ICU.[2] The strain of the virus, patient comorbidities, and age are some of the prognostic factors for disease severity.[3–5] Comorbid hypertension is often associated with the incidence of COVID-19.[6]

Several regions in Indonesia outside Java have a high proportion of people with hypertension (≥ 30% of the adult population)[7]. Through October 2022, an increase in COVID-19 cases and fatality rates were more than the national average observed in these areas, for example, in Central Sulawesi and South Sumatra, with rates of 2.8% and 4%, respectively.[8–10]

Angiotensin-converting enzyme (ACE) is a crucial component of the renin-angiotensin system (RAS), which that regulates blood pressure by converting angiotensin I to angiotensin

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II.[11] Furthermore, the RAS is involved in the pathogenesis of COVID-19: SARS-CoV-2 binds to angiotensin-converting enzyme-2 (ACE2), which is the homolog of ACE, in order to enter host cells.[12] This leads to a downregulation of tissue ACE2, leading to an increase in Ang II, as it cannot perform its role in offsetting the effects of ACE in the RAS.[13] Thus, SARS-CoV-2 infection affects the blood pressure system and facilitates the development of multiorgan damage from SARS-CoV-2.[14,15]

One of the factors associated with hypertension is family history. Genetic factors can increase blood pressure by 30-50%.[16] Variations in the *ACE* gene can cause variability in serum ACE levels and generate high ACE activity. Such genetic variations are known to explain susceptibility to hypertension.[17,18] One *ACE* gene polymorphism is characterized by the insertion (I) or deletion (D) of a 287-bp Alu sequence in intron 16 and a single nucleotide polymorphism (SNP) at multiple *ACE* gene loci.[19] Gomez (2020) found that sex (male), hypertension, hypercholesterolemia, and the *ACE* deletion-deletion (DD) genotype were significantly associated with the severity of COVID-19 in a Caucasian population.[20] In this study, we evaluated the genetic profiles of Indonesian people represented by two distinct regions, i.e., Central Celebes, East Indonesia, and South Sumatra, West Indonesia. Our results add valuable information for a more comprehensive evaluation of Southeast Asian data and provide a reference for individualized treatment and early detection to reduce SARS-CoV-2 transmission and protect vulnerable high-risk patients, particularly immunocompromised individuals, as well as members of conflict-affected population groups. Additionally, these results will provide insights to improve the prognosis of patients with COVID-19 in order to optimize outcomes.

MATERIALS AND METHODS

Subjects

This study was conducted based on the principles of the 1975 Declaration of Helsinki and approved by the Ethics Committee of each participating institution (Ethical Committee of the Faculty of Medicine, Tadulako University, and the Ethics Committee of the University of Indonesia Hospital, RSUI) number 7916/UN.28.1.30/KL /2020 and 0058/SKPE/KKO/2021/00. Written informed consent was obtained before the investigation. For this cross-sectional study, the Slovin formula was used to calculate the minimum necessary sample size, and a total of 186 subjects were enrolled: 91 residents of Palu City, Central Celebes, and 95 residents of Lahat District, South Sumatra, regions representing East Indonesia and West Indonesia, respectively. Furthermore, the samples obtained were divided into two groups: 91 subjects with no history of being infected with COVID-19, forming the non-COVID-19 group, and 95 subjects who were infected with COVID-19. The non-COVID-19 group comprised patients who received treatment for conditions other than COVID-19 in regional hospitals. A COVID-19 diagnosis was made based on a positive real-time polymerase chain reaction (rt-PCR) test for SARS-CoV-2 from nasopharyngeal swab samples of patients who received treatment at a regional hospital or self-quarantined under health center monitoring in the period from June to December 2021. All subjects met the following inclusion criteria: (1) they were at least 18 years old, and (2) they had completed medical records. The exclusion criteria were as follows: (1) immunocompromised patients, (2) patients under treatment for malignancy, (3) patients discharged at their own request, and (4) patients lost to follow-up. Clinical classification of patients was based on the COVID-19 treatment guidelines, i.e., (1) mild type: only mild clinical symptoms without signs of pneumonia on imaging; (2) moderate type: fever complications, respiratory symptoms, and pneumonia features; and (3) severe type: complicated by either of the following: (a) respiratory distress, with a respiratory rate of 30 breaths/min; or (b) mean oxygen saturation <93% at rest.[21] Comorbid hypertension was determined based on the diagnosis in the medical record and the presence of antihypertensive therapy received by the subject.

DNA extraction

A 5 mL venous blood sample was collected from each subject in an EDTA-containing tube and processed according to the QIAamp DNA Blood Mini Kit (QIAGEN, Hilden, Germany) manufacturer's protocol. Prior to use, the quality of the DNA was analyzed using agarose gel electrophoresis and GelRed nucleic acid gel staining (Biotium, Fremont, USA); the gels were observed using a Biometra Ti1 UV transilluminator (Biometra, Ltd., Jena, Germany), and the DNA was assessed using a NanoDrop™ One Microvolume UV–Vis Spectrophotometer Thermo Scientific™ (Thermo Fisher Scientific Inc., Waltham, USA).

I/D genotype analysis (rs1799752) in intron 16

To observe the I/D polymorphism, PCRs were carried out using genomic DNA (gDNA) as a template. A pair of specific primers synthesized by IDT (Integrated DNA Technologies, Inc. Coralville, USA) was used, i.e., the ACE I_D forward primer 5'-CTGGAGACCACTCCCATCCTTTCT-3' and the reverse primer 5'-GATGTGGCCATCACATTCGTCAGAT-3'. Approximately 25 µl of the amplification reaction mix was prepared from the following components: 10 µL ddH_2O, 12.5 µL MyTaq HS Red Mix, 0.75 µL of 20 µM of ACE forward primer, 0.75 µL of 20 µM primer ACE reverse, and 20-25 ng of DNA template. Amplification was performed by employing MyTaq™ HS Red Mix (Meridian, Memphis, USA) with a PCR T100™ Thermocycler (Bio-Rad Laboratories, Inc. California, USA) and T-Professional Basic Thermocycler (Biometra, Ltd., Jena, Germany) under the following conditions: 95°C for 1 minute (pre-denaturation), followed by 30 cycles of 94°C for 30 seconds (denaturation), 58°C for 15 seconds (annealing), 72°C for 30 seconds (extension) and 72°C for 6 minutes (post-extension).

The PCR products were analyzed on a 2% agarose gel stained with GelRed nucleic acid stain, with a 100-bp DNA ladder for reference (*Geneaid* Biotech Ltd. New Taipei City, Taiwan). The gel was observed using a gel imaging system to visualize the amplicon bands. Based on the bands that appeared on the gel at 190 bp and 490 bp, representing deletion and insertion,

respectively, we identified the genotypes II, ID, and DD and the alleles I and D.

Data and instruments

Data were collected from medical records, and validated instruments were distributed to each subject to be completed with a team of assistants. The variables included sex, height and weight, and smoking status. Category of blood pressure was defined according to European Society Hypertension (ESH) guidelines where the systolic and diastolic for normotensive is 120-129 and/or 80-84 mmHg; prehypertension is 130-139 and/or 85-89 mmHg; hypertension is ≥140 and/or ≥90 mmHg.[22] The GFR value was calculated using the formula described by Cockcroft and Gault in 1976 (mL/min/1.73 m^2). The prognostic category of acute kidney injury was defined according to the Kidney Disease: Improving Global Outcomes (KDIGO) guidelines.[23] Body mass index (BMI) was calculated as follows: BMI= weight(kg)/height(m^2). The BMI categories refer to nutritional status based on WHO guidelines.[24] Subjects were asked about their smoking status. Smokers were defined as smoking daily for more than one year or having quit less than six months ago. Nonsmokers were defined as having never smoked or having quit smoking more than six months ago.

Statistical analysis

Categorical variables were expressed as sums and percentages, while continuous variables were expressed as the mean and standard deviation ranges after the Shapiro–Wilk test to assess the normal distribution. Differences between groups were evaluated using chi-square and Fisher's tests. Bivariate logistic regression and odds ratios were used to test the strength of the risk. For all statistical tests, we used SPSS 21 (IBM Corp., Armonk, NY, USA). Statistical significance was defined as $p < 0.05$, and all p values were two-tailed.

RESULTS

Detection of *ACE* I/D gene polymorphisms

One hundred eighty-six subjects who met the criteria and were willing to participate in the study were enrolled. Blood samples were successfully collected, and gDNA was isolated and used as the template for PCR. The quality of the gDNAs was analyzed using agarose gel electrophoresis; the gel was stained with GelRed nucleic acid stain and observed using a UV transilluminator (data not shown). The concentration and purity of the gDNAs were also measured with a NanoDrop before PCR (data not shown).

The PCR results for the I/D genotypes at rs1799752 of the *ACE* gene showed the presence of a 490-bp fragment, which corresponded to a homozygous insertion-insertion (II) mutant genotype, i.e., an insertion of 287 bp in both alleles. In addition, a 190-bp fragment characterized as wild type corresponded to the DD genotype, and two fragments at 190 bp and 490 bp represented the heterozygous insertion–deletion (ID) mutant genotype (Figure 1). Genotype results were obtained for all 186 subject samples tested by using PCR; the II genotype was identified in 98 subjects (49%), the ID genotype in 71 subjects (42%), and the DD genotype in 17 subjects (9%) (Figure 2).

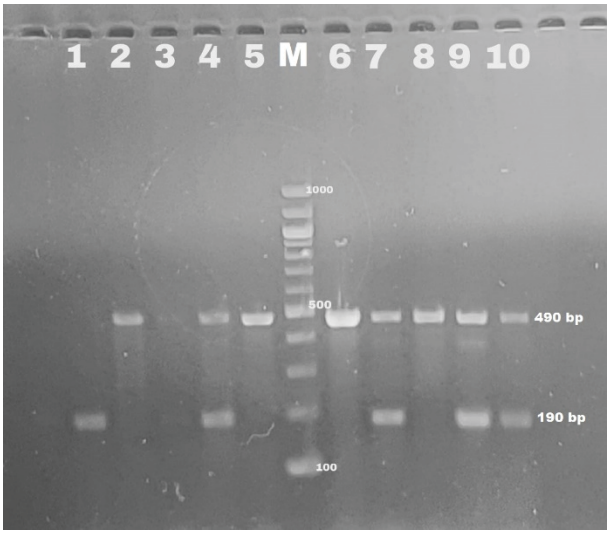

Figure 1. Representation of an I/D PCR gel photo. Lane 1 corresponds to the DD genotype with a 190-bp band. Lanes 2, 5, 6, and 8 correspond to the II genotype with 490-bp bands. Lanes 4, 7, 9, and 10 correspond to the ID genotype with bp bands of 490+190 bp. M, 100-bp DNA ladder (Geneaid).

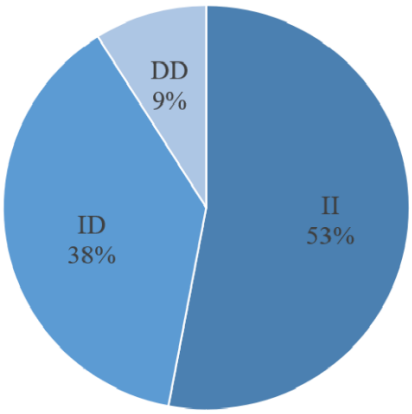

Figure 2. Proportion of ACE gene I/D genotype (rs1799752) frequencies in 186 subjects. A third genotype appeared in this population, with the most common genotype being II.

Distribution of the clinical characteristics and genotypes of all subjects with COVID-19

Based on their medical record histories, 95 subjects with confirmed COVID-19 were assessed, while 91 subjects who had never been diagnosed with COVID-19 were recruited as the non-COVID-19 group. Comparative analysis showed that the I/D genotypes of the *ACE* gene, hypertension, and high blood pressure were not significantly associated with a person's susceptibility to COVID-19 ($p > 0.05$). These data are shown in Table 1. Nevertheless, the percentages of the homozygous II and DD genotypes were higher in the COVID-19 group than in the non-COVID-19 group, whereas that of allele I was equal.

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Furthermore, the hypertension status of the non-COVID-19 group was higher than that of the COVID-19 group.

Clinical characteristics and distribution of the *ACE* gene I/D genotypes in the COVID-19 population

The COVID-19 subjects from the Lahat District, South Sumatra, showed a higher percentage of the DD genotype than those from Palu City, Central Celebes. In contrast, the ID genotype was more common in the Palu City population. Regarding allele frequencies, 70% of the Palu City population had allele I, compared to 63% of the Lahat District population. The percentage of people with comorbid hypertension, high blood pressure, and moderate to severe COVID-19 was significantly higher in the Palu population than in the Lahat population ($p<0.05$) (Table 2).

Clinical characteristics and *ACE* gene I/D genotype distribution in patients with comorbid hypertension and COVID-19

Hypertension is known to be a common comorbidity of COVID-19. This study found that 28% of COVID-19 subjects had comorbid hypertension (Table 1). Table 3 shows that the I/D genotype of the *ACE* gene is not a risk factor for comorbid hypertension or the severity of COVID-19. Middle-aged adults more at risk of developing hypertension than young adults. The blood pressure classification results indicated that 46% of patients with COVID-19 and hypertension had high blood pressure, and comorbid hypertension was a risk factor for more severe COVID-19 (Table 3).

Table 4 shows that hypertension and moderate to severe disease were risk factors for hospitalization ($p < 0.05$). We noted that the various clinical symptoms observed in the subjects were related to COVID-19. The proportion of COVID-19 cases based on symptoms is summarized in Figure S1. Furthermore, consistent with previously reported results, we noted two symptoms that were significantly associated with comorbid hypertension and COVID-19 severity, i.e., anosmia and dyspnea ($p < 0.05$). Dyspnea was predominant in patients with comorbid hypertension and moderate to severe COVID-19. Anosmia was observed in subjects without comorbid hypertension and mild COVID-19. The 67 hospitalized subjects were assessed based on routine laboratory parameters, including hematology, electrolytes, and biochemical parameters. The complete analysis of symptoms and laboratory parameters is summarized in Table S1. Based on vital signs and laboratory parameters, we found that decreased oxygen saturation and increased respiration rate were clinical conditions associated

Table 1. Distribution of the demographic profiles and ACE gene I/D genotype frequencies in COVID-19 and non-COVID-19 subjects

Variable	All COVID-19	Non-COVID-19 n (%)	p
ACE Genotype			
II	54 (57)	44 (48)	0.128
ID	30 (32)	41 (45)	
DD	11 (12)	6 (7)	
Sex			
Male	47 (49)	40 (44)	0.272
Female	48 (51)	51 (56)	
Age group (y)			
18-39	34 (35)	22 (24)	0.224
40-59	50 (53)	57 (63)	
>=60	11 (12)	12 (13)	
Comorbidity			
With Hypertension	27 (28)	46 (51)	0.003
Without Hypertension	68 (72)	45 (49)	
Blood Pressure Classification			
Normotension	63 (66)	43 (47)	0.000
Prehypertension	18 (19)	11 (12)	
Hypertension	14 (15)	37 (41)	
BMI (kg/m2)			
< 18.5	4 (4)	7 (8)	0.385
18.5–24.9	39 (41)	42 (46)	
≥ 25	52 (55)	42 (46)	
Smoking			
No	80 (84)	84 (92)	0.138
Yes	15 (16)	7 (8)	

I (Insertion), D (Deletion), BMI (Body Mass Index)

Table 2. Comparison of the frequencies of the ACE gene I/D genotypes, comorbid hypertension, and COVID-19 severity level in the Lahat District, South Sumatra, and Palu city, Central Sulawesi

Variable	Palu City - Central Celebes Subject n (%)	Lahat District - South Sumatera Subject n (%)	p
ACE Genotype			
II	25 (56)	29 (58)	0.610
ID	16 (36)	14 (28)	
DD (wild type)	4 (9)	7 (14)	
Allele Frequencies			
I	50 (70)	58 (63)	N/A
D	36 (30)	28 (37)	
Comorbidity			
With Hypertension	20 (44%)	7 (14%)	0.002
Without Hypertension	25 (56%)	43 (86%)	
Blood Pressure Classification			
Normotension	22 (48%)	38 (76%)	0.017
Prehypertension	12 (27%)	8 (16%)	
Hypertension	11 (25%)	4 (8%)	
Severity Level			
Mild	9 (20%)	27 (54%)	0.001
Moderate-Severe	36 (80%)	23 (46%)	

I (Insertion), D (Deletion)

Table 3. Association of the ACE gene I/D genotypes and patient demographics with comorbid hypertension and COVID-19 severity level

Variable	Comorbid Disease		p (OR 95% IC)	Severity Level		p (OR 95% IC)
	No Hypertension n (%)	Hypertension n (%)		Mild n (%)	Moderate-Severe n (%)	
ACE Genotype						
II	36 (53)	18 (67)	0.330 2.25 (0.44-11.52)	20 (56)	34 (58)	0.286 2.04 (0.55-7.55)
ID	23 (34)	7 (26)	0.725 1.37 (0.24-7.88)	10 (28)	20 (34)	0.223 2.40 (0.59-9.82)
DD (wild type)	9 (13)	2 (7)	Ref	6 (16)	5 (8)	Ref
Sex						
Male	33 (49)	14 (52)	0.948 0.88 (0.36-2.14)	16 (44)	31 (53)	0.579 0.72 (0.31-1.66)
Female	35 (51)	13 (48)		20 (56)	28 (47)	
Age group (y)						
18-39	30 (44)	4 (15)	Ref	15 (42)	19 (32)	Ref
40-59	32 (47)	18 (67)	0.023 0.16 (0.03-0.78)	17 (47)	33 (56)	0.479 1.53 (0.63-3.75)
≥60	6 (9)	5 (19)	0.559 0.68 (0.18-2.52)	4 (11)	7 (12)	0.919 1.38 (0.34-5.62)
BMI (kg/m2)						
< 18.5	1 (1)	3 (11)	0.075 0.12 (0.01-1.23)	2 (6)	2 (3)	0.562 1.44 (0.18-11.3)
18.5–24.9	29 (43)	10 (37)	Ref	16 (44)	23 (39)	Ref
≥ 25	38 (56)	14 (52)	1.000 1.07 (0.41-2.73)	18 (50)	34 (58)	0.684 1.31 (0.56-3.09)
Comorbidity						
With Hypertension	N/A		N/A	4 (11)	23 (39)	0.007 5.11 (1.59-16.36)
Without Hypertension				32 (89)	36 (61)	
Blood Pressure Classification						
Normotension	52 (76)	8 (28)	Ref	23 (64)	37 (63)	Ref
Prehypertension	14 (21)	6 (21)	0.000 0.02 (0.00-0.13)	9 (25)	11 (19)	0.792 0.76 (0.27-2.11)
Hypertension	2 (3)	13 (46)	0.003 0.07 (0.01-0.39)	4 (11)	11 (19)	0.588 1.71 (0.49-6.01)

I (Insertion), D (Deletion), BMI (Body Mass Index)

Table 4. Association of clinical characteristics with comorbid hypertension and severity of COVID-19

Variable	Comorbid Disease		p (OR 95% IC)	Severity Level		p (OR 95% IC)
	No Hypertension n (%)	Hypertension n (%)		Mild n (%)	Moderate-Severe n (%)	
Type of Care (n = 95)						
Hospital Admission	42 (62)	25 (93)	0.006 0.13 (0.03-0.59)	9 (25)	58 (98)	0.000 0.01 (0.00-0.05)
Self-quarantine	26 (38)	2 (7)		27 (75)	1 (2)	
LoT (≥ 14 days)	10 (15)	5 (18)	0.429 1.32 (0.41-4.29)	4 (11)	11 (19)	0.492 1.83 (0.53-6.26)
Symptom (n = 95)						
Anosmia	32 (47)	5 (19)	0.019 0.26 (0.09-0.75)	22 (59)	15 (41)	0.001 0.22 (0.09-0.53)
Dyspnea	24 (35)	20 (74)	0.001 5.24 (1.94-14.15)	8 (18)	36 (82)	0.000 5.48 (2.13-14.01)
Vital Sign (n = 67)						

Temperature (> 37°C)	9 (21)	2 (8)	0.136 0.32 (0.06-1.61)	1 (10)	10 (90)	0.543 1.67 (0.19-14.85)
Oxygen Saturation (< 95%)	12 (28)	9 (36)	0.718 1.41 (0.49-4.04)	0 (0)	21 (100)	0.026 1.57 (1.29-1.90)
Respiration Rate (> 20 bpm)	19 (45)	14 (56)	0.549 1.54 (0.57-4.17)	1 (3)	32 (97)	0.015 9.85 (1.15-83.88)
Pulse (> 100 bpm)	9 (21)	7 (28)	0.754 1.42 (0.45-4.47)	2 (13)	14 (87)	0.634 1.11 (0.21-5.99)
Biochemical Tests (n = 65)						
ALT (> 45 U/L)	6 (15)	7 (29)	0.149 2.33 (0.68-8.03)	2 (15)	11 (85)	0.589 0.87 (0.16-4.81)
AST (> 35 U/L)	12 (29)	12 (50)	0.160 2.42 (0.85-6.87)	2 (8)	22 (92)	0.277 2.26 (0.43-11.91)
Creatinine (> 1.1 mg/dL)	10 (24)	13 (54)	0.031 3.66 (1.25-10.72)	4 (17)	19 (83)	0.397 0.64 (0.15-2.67)
GFR (mL/min/1.73m2)						
Stage 3 (30–59)	7 (17)	9 (36)	N/A	3 (19)	13 (81)	N/A
Stage 4 (15–29)	1 (2)	1 (4)		0 (0)	2 (100)	
Stage 5 (< 15)	1 (2)	1 (4)		1 (50)	1 (50)	
Blood Glucose (>200 mg/dL)	4 (10)	7 (28)	0.049 3.71 (0.95-14.39)	2 (18)	9 (82)	0.485 0.68 (0.12-3.85)

LoT (length of treatment), ALT (alanine aminotransferase), AST (aspartate aminotransferase, GFR (glomerular filtration rate)

with moderate to severe COVID-19. In addition, we found that increased creatinine and blood glucose values were associated with comorbid hypertension in COVID-19 patients.

DISCUSSION

This study provides information on the distribution of *ACE* gene I/D genotypes and their correlation with demographic profiles and clinical characteristics, as well as their association with the severity of COVID-19 and comorbid hypertension in an Indonesian population. This study also presents data on SARS-CoV-2 infection in Indonesia, particularly in regions of West Indonesia and East Indonesia. I/D gene polymorphisms of the 287-bp Alu element often occur in intron 16 of the *ACE* gene.[25,26] Although this insertion occurs in the intron region, it has been reported that ACE exonization appears based on splicing analysis. Thus, the presence of an exonized Alu element extends the length of this series of segments, and upon translation, the sequence exhibits a premature termination codon (PTC), indicating a possible truncation of the protein.[27]

In this study, all dominant subjects had the II genotype (53%), although the difference in the distribution of I/D genotypes of the *ACE* gene between the COVID-19 and non-COVID-19 groups was nonsignificant (p= 0.128). These data showed that the I/D genotypes were not associated with an individual's susceptibility to COVID-19. Similar results were also shown in the studies by Gomez (2020) and Hubacek (2021), which did not show a significant difference.[20,27] However, Jacob's (2021) study on the levels of ACE2 protein in the pulmonary alveolar epithelium based on I/D genotypes stated that patients with genotype II showed an increase in the level of ACE2 protein in the lung epithelium, which can facilitate the entry of the virus into the host organism, given that the virus uses the ACE2 receptor as an entrance.[28] Mutations at other points in the *ACE* gene, mutations in other genes that play a role in the RAS, and epigenetic factors can affect the phenotype and gene expression in each population. Our previous research showed that hypertension and the genes rs4331 ACE and rs2074192 ACE2 impact the severity of COVID-19.[18,29] Therefore, differences in results between populations could be due to the interaction of other factors that affect individual susceptibility to hypertension and COVID-19. We assume that at the gene level, there is a role for *ACE* gene mutations at other loci, that RAS-related genes are involved as well, and that epigenetic factors, namely, DNA methylation and histone modifications, also influence the phenotype. On the other hand, lifestyle, environmental exposure, and social status can also change gene expression.[30,31]

As an archipelagic country with heterogeneous characteristics and lifestyles, Indonesia has a diverse population. In particular, the people of western Indonesia, e.g., the Lahat District, are ethnic Deutero-Malays, and the people of eastern Indonesia, e.g., Palu City and Central Celebes, are ethnic Proto-Malays and Melanesians; these populations are interesting groups to study. Here, we report no significant difference in the distribution of the *ACE* I/D genotypes between the two regions. The percentage of the II genotype in the two areas was comparable, and the ID genotype was more common in the Palu population. In contrast, the DD genotype was more common in the Lahat population. Comorbid hypertension was found more frequently in the Palu population than in the Lahat population, i.e., 44% and 14%, respectively. This result is consistent with the higher percentage of high blood pressure

in the Palu population. Based on the 2018 data of Riskesdas, Central Celebes ranks 11th among the regions with the highest prevalence of hypertension based on doctors' diagnoses, and the South Sumatra region ranks 24th.[7] We also found that the Palu population tended to show more severe COVID-19 symptoms than the Lahat population; therefore, hypertension was assessed as a risk factor for the severity of COVID-19.

In an attempt to compare regions, a previous study on a Malaysian male population concluded that 59% of hypertensive patients had the DD genotype.[32] As illustrated in Figure S2, several studies have also concluded that the DD genotype is related to the severity of COVID-19. In our study, there was no significant relationship between the I/D genotypes of the *ACE* gene and the incidence of hypertension and severity of COVID-19 ($p > 0.05$). These results align with Gunal's (2021) study, which did not find an association between the I/D genotypes of the *ACE* gene in patients and the severity of COVID-19.[33] However, we found a higher percentage of the II genotype in the group with comorbid hypertension and moderate to severe COVID-19 susceptibility. In comparison, the II genotype is associated with more severe COVID-19 in European populations.[27,34] The II genotype is also related to low ACE activity.[35] The association between ACE and ACE2 may confer susceptibility to SARS-CoV-2 infection and COVID-19.[28]

Hypertension is still the most common comorbidity in COVID-19 patients to date. As illustrated in Figure S3, COVID-19 with comorbid hypertension was more severe than COVID-19 without hypertension in Asian and European subjects. We found that 28% of subjects presented with comorbid hypertension, and middle age was an associated risk factor. A study in South Korea showed that the 50- to 59-year-old age group was the group most likely to be hospitalized for COVID-19, and 30% of hospitalized patients exhibited comorbid hypertension.[36] Research involving residents of Wuhan also showed that those over 40 years of age experienced more severe COVID-19 symptoms,[37] as illustrated in Figure S4. It was previously reported that older age was a significant independent predictor of mortality in SARS and MERS.[38]

Our analysis showed that subjects with comorbid hypertension had a fivefold increase in the risk of moderate to severe COVID-19 symptoms, and 93% of hypertensive subjects required hospitalization. In addition, other studies have shown that hypertension is the most common cause of death by disease in the Malaysian population.[39] The frequency of the II genotype is higher in East Asian countries and lower in European and African countries.[40,41] Different I/D polymorphisms alter circulating concentrations of ACE in tissues. SARS-CoV-2 infection disrupts the balance of ACE and ACE2 and increases proinflammatory and profibrotic responses.[42-44] This reaction can increase the tendency for more severe COVID-19 in people with hypertension. This is consistent with our result that complaints of shortness of breath are supported by a decrease in oxygen saturation and an increase in the respiratory rate in the vital signs of subjects with comorbid hypertension and moderate to severe COVID-19. This inflammatory response can rapidly progress to acute respiratory distress syndrome (ARDS).[43,45,46] Aung (2020) stated that *ACE* gene polymorphisms could determine the risk of ARDS and death from sepsis, and the II genotype significantly favored survival until day 28 in patients with ARDS ($p=0.03$).[40]

This study found that patients with symptoms of anosmia tended to experience mild symptoms overall, whereas patients with dyspnea tended to experience moderate to severe symptoms. Mutia (2021) stated that the prevalence of anosmia in COVID-19 patients was 38.2%, and the prevalence was 10.2 times higher in COVID-19 patients than in patients with other respiratory infections.[47] This result is assumed to be due to the defense mechanism of supporting cells, such as sustentacular cells, as the initial site of SARS-CoV-2 infection in the olfactory epithelium.[47] ACE2 is expressed in almost all human organs to varying degrees as type II alveolar epithelial cells in the respiratory system, suggesting that the lung is the main target of SARS-CoV-2. In addition, ACE2 is highly expressed in myocardial, pancreatic, and renal proximal tubular cells and small intestinal enterocytes.[48,49] Autopsies of SARS patients have shown that SARS-CoV infection causes injury to several organs, such as the kidneys and liver.[50] These results align with the abnormal laboratory values in this study, such as elevated blood glucose creatinine, AST, and ALT values. Other studies have shown that many critically ill COVID-19 sufferers experience organ damage, including acute lung injury, acute kidney injury, liver dysfunction, and pneumothorax.[51–53] The Harapan (2022) meta-analysis study also found that COVID-19 patients with acute liver injury (ALI) had a higher risk of developing severe COVID-19 than patients without ALI.[54] Organ involvement and injury are closely related to the distribution of receptors in the body. The presence of a virus-induced inflammatory response, including overexpression of cytokines and chemokines, excessive recruitment of inflammatory cells, lack of interferon, and possible autoantibody production, is considered a vital factor in disease pathogenesis. These findings suggest that abnormal laboratory values indicate severe COVID-19.[15,55] In conclusion, it can be assumed that in our studied population, *ACE* gene I/D polymorphisms and hypertension are not associated with susceptibility to SARS-CoV-2 infection, but the presence of comorbid hypertension is a risk factor for more severe COVID-19.

This study has several limitations. First, this was a cross-sectional study; therefore, we could not directly monitor the condition of the COVID-19 patients. We conducted this study before the vaccination target was fully achieved; the results would likely have been different if the target had been reached. Finally, the number of patients willing to participate in the study was limited, making it difficult to generalize the results of this *ACE* gene polymorphism analysis to other ethnic groups. However, our results can serve as a reference and comparison for further assessing the association of *ACE* I/D gene polymorphisms in a wider study population.

ACKNOWLEDGMENTS

We would like to thank Nadia Farhanah Syafhan, Ph.D for contributing to the Lahat sample analysis of this study. We

thank the COVID-19 nursing and laboratory staff of the regional hospitals at Lahat and Palu for contributing to this study.

DATA AVAILABILITY

The datasets used and analyzed during the current study are available from the corresponding author upon reasonable request.

AUTHORS CONTRIBUTION STATEMENT

Conceptualization: AM, RA, SIW, ES; Data curation: IF, DM, LA, SK; Formal analysis: IF, DM; Methodology: AM, IF, DM; Writing –original draft: IF, DM, LA, SK; Writing –review & editing: AM, IF

CONFLICTS OF INTEREST

The authors declare no competing interests.

FUNDING

This research was funded by Universitas Indonesia World Class University Research Grants for International Publication Q1 (PPI Q1) 2021, No.: NKB-521/UN2.RST/HKP.05.00/2021 to AM, partially funded by Publikasi Terindeks Internasional Q2 (PUTI Q2) 2022-2023, No.: NKB-543/UN2.RST/HKP.05.00/2022 to AM.

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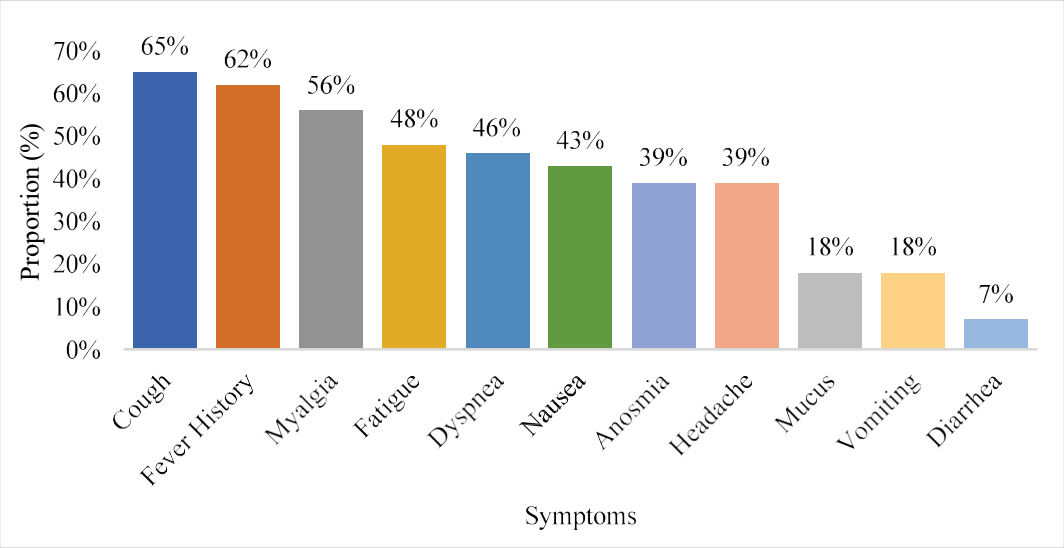
www.pharmacypractice.org (eISSN: 1886-3655 ISSN: 1885-642X)

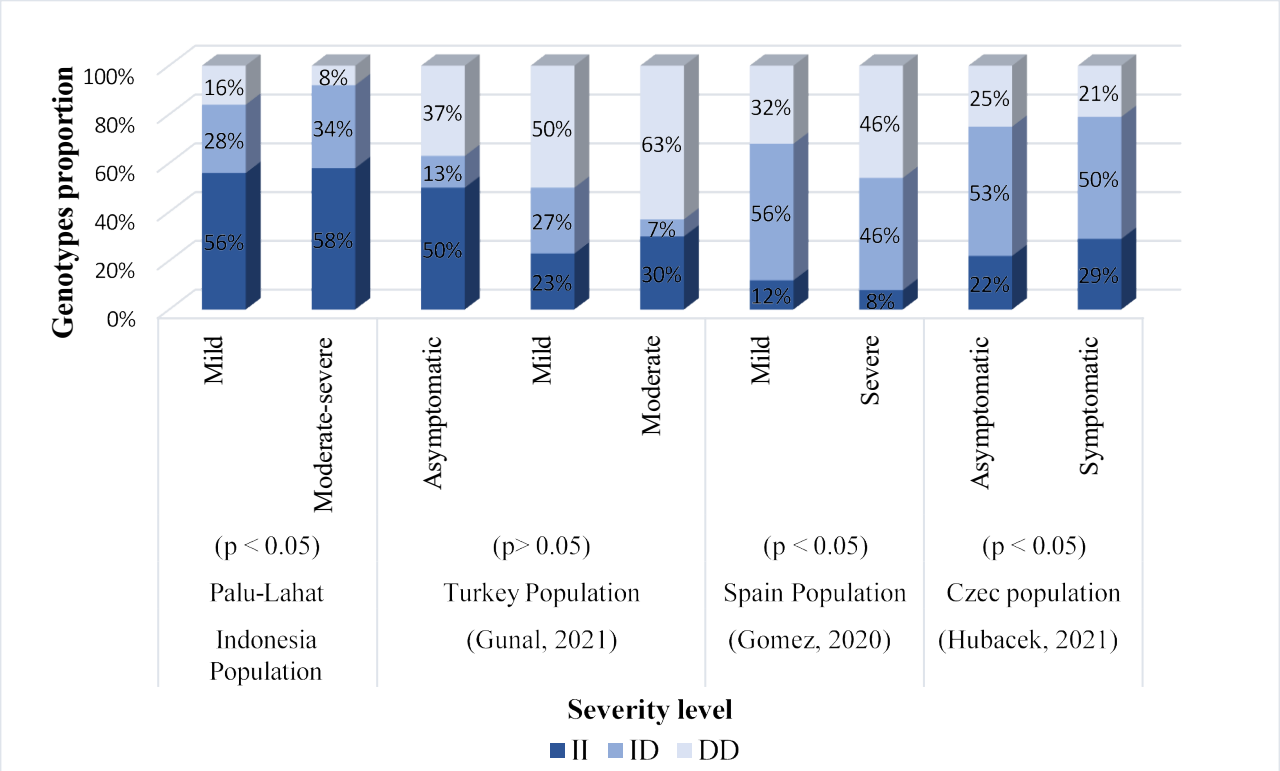
Supplementary Table 1. Association of clinical characteristics with comorbid hypertension and severity of Covid-19

Comorbid disease				**Severity level**		
Variables	**Non-Hypertension n (%)**	**Hypertension n (%)**	**p (OR 95% IC)**	**Mild n (%)**	**Moderate-Severe n (%)**	**p (OR 95% IC)**
Symptom (n = 95)						
Cough	43 (63)	19 (70)	0.675 1.38 (0.53-3.61)	20 (32)	42 (68)	0.183 1.98 (0.83-4.69)
Fever History	46 (68)	13 (48)	0.125 0.44 (0.18-1.10)	23 (39)	36 (61)	0.951 0.88 (0.37-2.09)
Myalgia	41 (60)	12 (44)	0.240 0.53 (0.21-1.29)	22 (42)	31 (58)	0.547 0.70 (0.30-1.64)
Fatigue	34 (50)	12 (44)	0.794 0.80 (0.33-1.96)	21 (46)	25 (54)	0.194 0.52 (0.23-1.22)
Dyspnea	24 (35)	20 (74)	0,001 5.24 (1.94-14.15)	8 (18)	36 (82)	0.000 5.48 (2.13-14.01)
Nausea	28 (41)	13 (48)	0.697 1.33 (0.54-3.25)	15 (37)	26 (63)	0.987 1.10 90.48-2.55)
Anosmia	32 (47)	5 (19)	0,019 0.26 (0.09-0.75)	22 (59)	15 (41)	0.001 0.22 (0.09-0.53)
Headache	28 (41)	9 (33)	0.636 0.71 (0.28-1.82)	16 (43)	21 (57)	0.521 0.69 (0.29-1.61)
Mucus	15 (22)	2 (7)	0.078 0.28 (0.06-1.33)	6 (35)	11 (65)	1.000 1.15 (0.38-3.42)
Vomiting	14 (20)	3 (11)	0.218 0.48 (0.13-1.83)	7 (41)	10 (59)	0.975 0.84 (0.29-2.46)
Diarrhea	7 (10)	0 (0)	0.088 0.69 (0.60-0.79)	5 (71)	2 (28)	0.070 0.22 (0.04-1.18)
Hematological test (n = 67)						
WBC (10^9/L) < 4 or > 11 (10^9/L)	13 (31)	7 (28)	1.000 0.87 (0.29-2.58)	4 (20)	16 (80)	0.255 0.48 (1.11-2.00)
RBC (< 4.1 or > 6.1.10^{12}/L)	7 (17)	2 (8)	0.269 0.43 (0.08-2.28	2 (22)	7 (78)	0.347 0.48 (0.08-2.79)
Haemoglobin (< 14 or > 18 g/dL)	11 (26)	4 (16)	0.506 0.54 (0.15-1.91)	3 (20)	12 (80)	0.321 0.52 (0.11-2.39)
Hematocrit (< 36 or > 47%)	14 (33)	7 (28)	0.855 0.78 (0.26-2.29)	4 (19)	17 (81)	0.292 0.52 (0.12-2.17)
Platelets (< 150 or > 450.10^9/L)	9 (21)	1 (4)	0.051 0.15 (0.02-1.29)	1 (10)	9 (90)	0.597 1.47 (0.16-13.2)
Electrolyte test (n = 64)						
Sodium (< 136 or > 146 mEq/L)	19 (48)	10 (42)	0.846 0.79 (0.28-2.19)	6 (21)	23 (79)	0.152 0.36 (0.08-1.59)
Potassium (< 3.5 or > 5 mEq/L)	14 (35)	4 (17)	0.196 0.37 (0.11-1.30)	2 (11)	16 (89)	0.508 1.44 (0.27-7.67)
Chloride (< 98 or > 106 mEq/L)	10 (25)	9 (38)	0.437 1.80 (0.60-5.37)	4 (21)	15 (79)	0.251 0.47 (0.11-1.98)
Biochemical test (n=65)						
ALT (U/L)	6 (15)	7 (29)	0.149 2.33 (0.68-8.03)	2 (15)	11 (85)	0.589 0.87 (0.16-4.81)
AST (U/L)	12 (29)	12 (50)	0.160 2.42 (0.85-6.87)	2 (8)	22 (92)	0.277 2.26 (0.43-11.91)
Creatinine (> 1.1 mg/dL)	10 (24)	13 (54)	0.031 3.66 (1.25-10.72)	4 (17)	19 (83)	0.397 0.64 (0.15-2.67)
GFR (mL/min/1.73m2)						
Stage 1 and 2 (≥ 60)	32 (79)	13 (54)	N/A	5 (11)	40 (89)	N/A

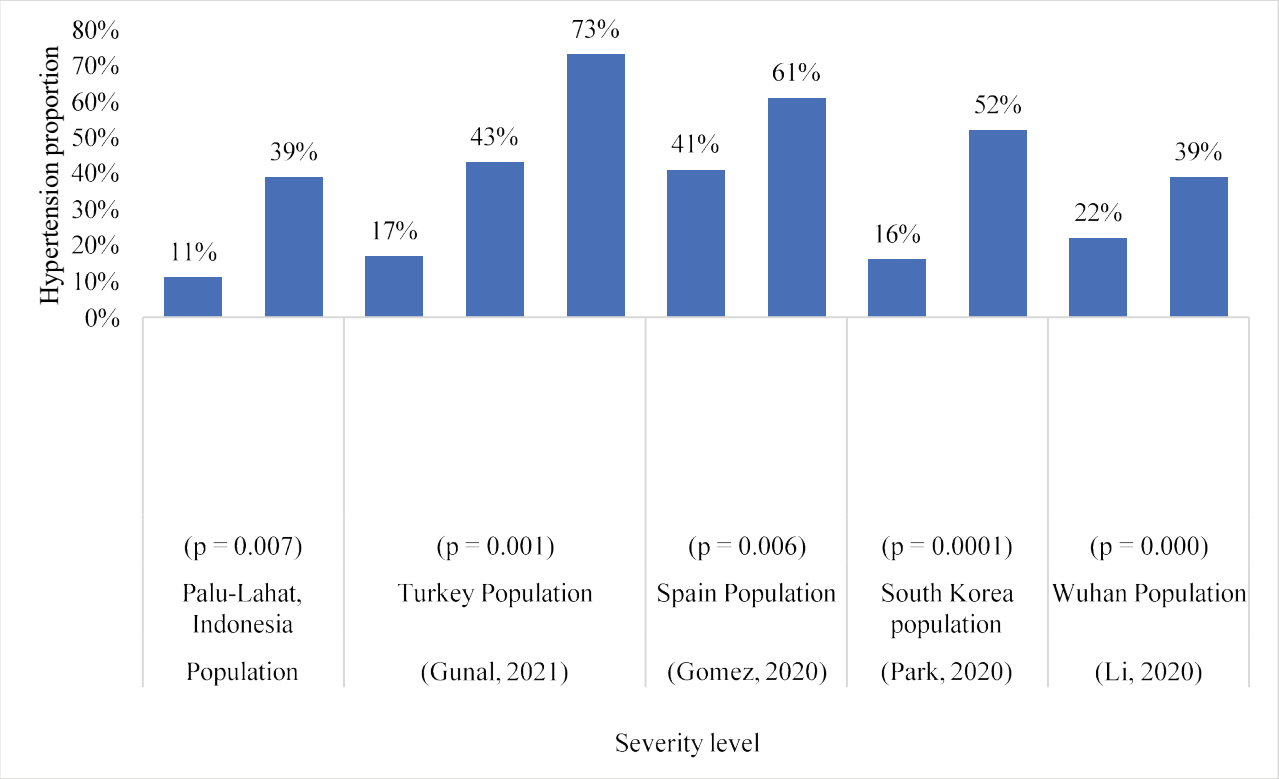
Stage 3 (30 - 59)	7 (17)	9 (38)		3 (19)	13 (81)	
Stage 4 (15 - 29)	1 (2)	1 (4)		0 (0)	2 (100)	
Stage 5 (< 15)	1 (2)	1 (4)		1 (50)	1 (50)	
Urea (> 50 mg/dL)	7	1	0.126 0.21 (0.02-1.83)	2	6	0.305 0.42 (0.07-2.50)
Blood Glucose (mg/dL)	4 (10)	7 (28)	0.049 3.71 (0.95-14.39)	2 (18)	9 (82)	0.485 0.68 (0.12-3.85)



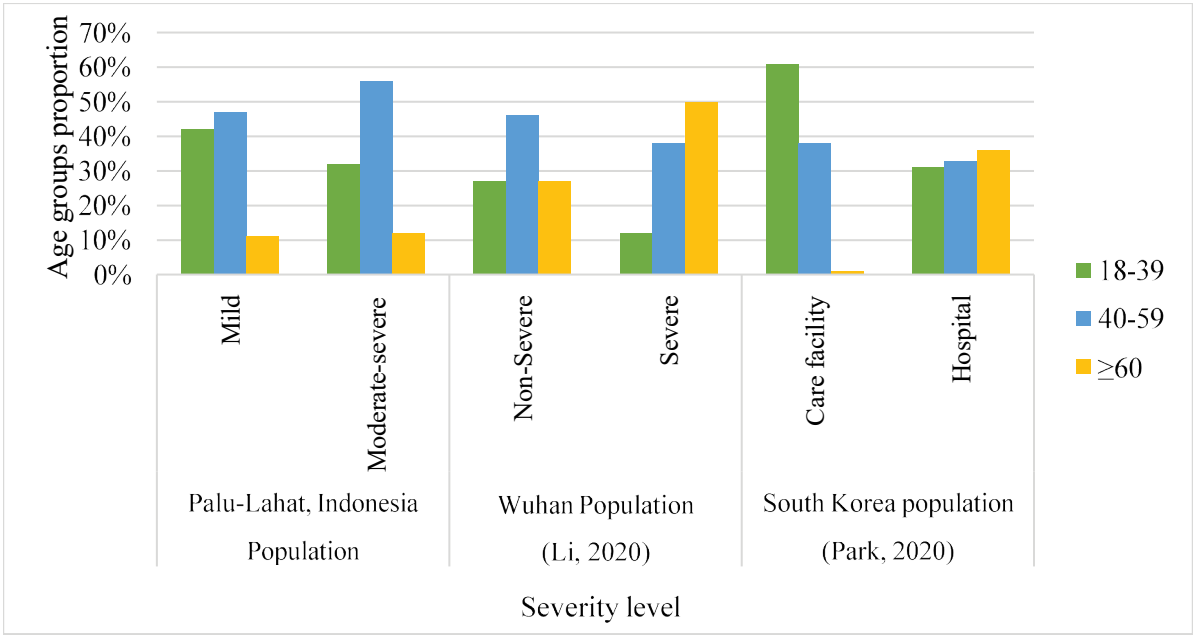
Supplementary Figure 1. The proportion of Covid-19 cases is based on symptoms



Supplementary Figure 2. Comparison of associations of the ACE gene ID genotype with Covid-19 severity in Asian and European populations



Supplementary Figure 3. Comparison of associations of comorbid hypertension with Covid-19 severity in Asian and European populations



Supplementary Figure 4. Comparison of age group percentages with Covid-19 severity and type of care in Asian populations