

# Angiotensin-converting enzyme 2 SNPs as the common genetic loci and optimal early identification genetic markers for COVID-19

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## Research Article

**Keywords:** ACE2, SNP, COVID-19, cardiovascular risks

**Posted Date:** November 1st, 2021

**DOI:** <https://doi.org/10.21203/rs.3.rs-1018735/v1>

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# Abstract

## Background

Angiotensin-converting enzyme 2 (ACE2) is implicated as a host cell receptor that causes infection in the pathogenesis of Coronavirus disease 2019 (COVID-19), and its genetic polymorphisms in the ACE2 gene may promote cardiovascular disease and systemic inflammatory injury in COVID-19. Hence, genetic background may potentially explain the broad inter-individual variation of disease susceptibility and/or severity.

## Methods

The genetic susceptibility to COVID-19 by examining single-nucleotide polymorphisms (SNPs) of ACE2 was analyzed in 196 patients with COVID-19 and 210 normal controls using TaqMan genotyping assay.

## Results

We demonstrated that ACE2 SNP rs4646142, rs6632677, and rs2074192 were associated with COVID-19 (all  $P < 0.05$ ), and the differences of ACE2 SNPs rs4646142 and rs6632677 were correlated with COVID-19 related systemic inflammatory injury and cardiovascular risk. Specially, rs4646142 was associated with high-sensitive C-reactive protein (hs-CRP), prealbumin (PAB), apolipoprotein A (APOA), high-density lipoprotein (HDL), and acid glycoprotein (AGP). Rs6632677 was also associated with elevated CRP and haptoglobin (HPT).

## Conclusions

Our results suggest that early identification of these individuals can provide a possible strategy for preventing the spread of the COVID-19, and ACE2 SNPs rs4646142 and rs6632677 may be a common genetic loci and optimal early identification genetic marker for COVID-19 with cardiovascular risks.

## 1. Background

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the virus that causes Coronavirus disease 2019 (COVID-19) via respiratory infection, was first discovered in Wuhan, China, in December 2019. Since then, the virus has become deeply entrenched in many countries and territories, and is an enormous threat to global public health and the economy. As of June 02, 2021, over 170 million cases of COVID-19 have been confirmed worldwide and 3.56 million deaths have resulted from SARS-CoV-2 infection (1). The most common clinical symptoms of COVID-19 are fever (2), dry cough, headache (3), sore throat, nausea, breathlessness, loss of taste or smell (4) while some remain asymptomatic (5, 6). Morbidity and mortality caused by COVID-19 are also closely associated with past co-existing diseases,

especially cardiovascular diseases(7). Meanwhile, human genetic factors may lead to the increased transmissibility of SARS-CoV-2 and contribute to persistent progressive disease observed in a part of infected people, but these genetic factors are largely unknown (8). Firstly, four mathematical models were constructed to predict SARS-CoV-2 transmission and the effectiveness of eradication strategies (9). The effective detection rate and detection time of SARS-CoV-2 real-time reverse transcriptase–polymerase chain reaction (rRT-PCR) analysis with the sensitivity and specificity of various antibody detection methods were reviewed (10). We have further investigated that the determinants of disease severity seem to be mainly derived from host factors, such as age and lymphopenia, while genetic variation of the virus has no significant effect on the outcomes of COVID-19 (11). Furthermore, abundant studies have indicated the possible associations of ACE2 single-nucleotide polymorphisms (SNPs) and cardiovascular risks in patients with cardiovascular disease (12–14). These observations suggest how much of the variation in COVID-19 disease severity may be explained by genetic types. Hence, early warning of inflammatory infections and cardiovascular disease risks by identifying host genetic DNA polymorphisms (variants), including excessive immune responses to viruses, will greatly promote the development of new prevention and/or treatment strategies for COVID-19.

At present, the cognition of the physicochemical property of SARS-CoV-2 mostly derived from research on SARS-CoV and MERS-CoV. As is well-known, angiotensin-converting enzyme 2 (ACE2) was highly expressed in human pneumocytes, intestinal epithelial cells and endothelial cells (15, 16). Given the importance of 3D structure of proteins in protein-protein interaction (PPI) it's not surprising that any process resulting in ACE2s' 3D structure change could influence COVID-19s' cell entry. Accumulating evidence has shown that 3D structure of ACE2 might be influenced at both transcriptional and post-transcriptional levels. SNPs are able to impact on protein function, structure, stability, and abundance (17). Therefore, the fundamental role of ACE2 in virus infection created this hypothesis that different complications to SARS-CoV-2 might be due to different ACE2 SNPs (18). Since the outbreak of the COVID-19 pandemic several authors have speculated about the role of the ACE and ACE2 gene polymorphisms in disease susceptibility and severity. However, as to what extent the variable response to complications (inflammatory infections and cardiovascular disease risks) with COVID-19 is influenced by the variability of the hosts' genetic background was almost unclear.

Using SNPs for DNA sequence comparisons are commonly used in genetic diversity and evolutionary studies, and it is especially helpful in identifying the mutated coronavirus genomes (19). Recently, ACE2 polymorphisms were mentioned in human population, and ACE2 expression could impact the susceptibility of people to cardiovascular, hypertension, dyslipidemia, diabetes and SARS-CoV-2 infection (20). For example, 8 ACE2 SNPs (rs2074192, rs233575, rs4240157, rs4646156, rs4646188, rs1978124, rs2048683, and rs879922) were related to Type 2 diabetes mellitus (T2D) (13). Furtherly, these ACE2 SNPs were correlated with diabetic related cardiovascular complications (13). The association of ACE2 (rs233575) gene polymorphisms with blood pressure was also found in adolescents (21). More discrepant outcomes to COVID-19 disease in connected with ACE2 polymorphisms (rs2074192) in obese, smoking males (21). Besides, two SNPs (K26R and S331F), observed missense variants, can reduce ACE2 receptor affinity for the spike (S) protein (22).

In this study, we speculated that genetic factors in the ACE2 gene are likely to impact the susceptibility to COVID-19 with previous coexisting disease. By establishing the SNP genotyping method and collecting literature to ACE2 polymorphisms, we chose rs2074192, rs6632677, rs4646142, rs2048683, rs4240157, five intronic SNP in ACE2, based on different genotyping, the analysis of clinical indicators is performed in COVID-19 patients. We verified the pathological degree of the COVID-19 was associated with hypertension and diabetes. Furtherly, our observations illustrated that ACE2 SNPs rs4646142 and rs6632677 may be an optimal genetic susceptibility marker for COVID-19 related cardiovascular complications. In summary, this study aims to present possible variants in the regulatory regions of ACE2, which may lead to marked inflammatory infections and cardiovascular disease clinical indicators variations in the prevalence and mortality of COVID-19. These may be further used in the genetic association study of patients with SARS-CoV-2 infection.

## 2. Methods

### 2.1. Clinical subjects and specimens

A total of 196 whole blood samples were collected from COVID-19 patients who were confirmed to have COVID-19 by qRT-PCR (Quantitative Real-time PCR) from the affiliated hospital Shanghai Public Health Clinical Center of Fudan University (Shanghai, China). A total of 210 whole blood samples were collected from normal subjects in the affiliated hospital Shanghai Public Health Clinical Center of Fudan University. The demographic and baseline clinical characteristics included smoking, drinking, allergy, surgery, previous coexisting disease. All procedures were reviewed and approved by the Ethics Committee of Shanghai Public Health Clinical Center, Fudan University, and have obtained written informed consents from all subjects.

### 2.2. Genomic DNA extraction

Peripheral blood (2 mL) was obtained from COVID-19 patients, and was stored in ethylenediaminetetraacetic acid tubes at -20°C until required. Genomic DNA (gDNA) was extracted from the peripheral blood samples using a TIANamp Blood DNA Kit (TIANGEN Biotech, Cat#: DP304-02, Beijing, China) according to the manufacturer's protocol. Finally, gDNA was dissolved in 70 µL RNA-Free water. gDNA integrity and concentration were assessed about 20-50 ng/µL using Nano Drop ND-2000 (ThermoFisher, Waltham, MA).

### 2.3. Genotyping assay

Five ACE2 SNPs (rs4646142, rs2048683, rs4240157, rs6632677, rs2074192) (ThermoFisher, Cat#: 4351379, Waltham, MA) were selected based on summarizing the literature and HapMap database. ACE2 genotyping was performed by TaqMan fluorescence probe assay (ThermoFisher, Waltham, MA). In total, 20 ng of gDNA per sample was used for reaction. We performed PCR in a total reaction volume of 5 µL, including 2.5 µL of 2 × Master Mix (ThermoFisher, Cat#: 4440038, Waltham, MA), 0.25 µL of 20 × TaqMan® Assays Buffer (ThermoFisher, Cat#: 4351379, Waltham, MA), 10 ng of gDNA, and ddH<sub>2</sub>O. The

reaction was initiated at 95°C for 10 min followed by 95°C (15s) and 60°C (1 min) for 40 cycles by using the LightCycler 480 II Instrument (Roche Molecular Systems, Inc).

## 2.4. Statistical analysis

Allele frequencies were calculated following the genotypes. categorical variables were presented as number (%) and continuous variables were presented as mean  $\pm$  standard deviation (SD). Categorical variables were compared using Chi-square-test and continuous variables were compared using one-way analysis of variance. The Odds ratio (OR) between control genotype and SARS-CoV-2 risk genotype for each ACE2 SNP among categorical variables was evaluated using binary logistic regression. a 95% confidence interval. A *P* value less than 0.05 was considered statistically significant. All probabilities are two-tailed.

## 3. Results

### 3.1. Characteristics of the study participants

Among 196 patients who had a laboratory-confirmed SARS-CoV-2 infection, there were two cases of mild type, 184 cases of common, three cases of critical disease, and seven cases of severe COVID-19 (Table 1), which were classified according to the latest WHO regulations (23). The correlation between the COVID-19 severity degree and baseline clinical characteristics were showed in Table 1. We found that the proportion of older patients ( $\geq 60$  years old) with critical and severe COVID-19 were higher than common ( $P = 0.029$ ). The prevalence of major coexisting illnesses such as heart disease ( $P = 0.021$ ) and Pulmonary disease ( $P < 0.001$ ) were also more likely to exist differently in these patients with COVID-19. Interestingly, lower percentage of patients with drug allergy were discovered in the patients with wild, critical disease and severe COVID-19. From the disease origin perspective, there was significantly different disease types of COVID-19 in the inland and overseas patient ( $P < 0.001$ ). The most-reported symptoms of COVID-19 were fever (84.2%), dry cough (48.9%) and are shown in Table 2. Furthermore, the most common initial therapies were oxygen and arbidol antiviral treatment (Table 2).

Table 1  
The baseline clinical characteristics of the study population.

Characteristics	Mild (n =2)	Common (n =184)	Severe (n =7)	Critical (n =3)	P value
Age, mean ± SD	18.50±3.536	49.01±14.36	60.14±15.19	66.33±7.371	< 0.001
<b>Distribution - n (%)</b>					<b>0.029</b>
<60	2 (100)	133 (72.3)	2 (28.6)	1 (33.3)	-
≥60	0 (0)	51 (27.7)	5 (71.4)	2 (66.6)	-
<b>Sex – n (%)</b>					0.303
Male	0 (0)	91 (49.5)	5 (71.4)	2 (66.6)	-
Female	2 (100)	93 (50.5)	2 (28.6)	1 (33.3)	-
<b>Smoking- n (%)</b>	0 (0)	12 (6.5)	1 (14.3)	0 (0)	0.796
<b>Drinking- n (%)</b>	0 (0)	15 (8.2)	1 (14.3)	0 (0)	0.851
<b>Food allergy- n (%)</b>	0 (0)	0 (0)	0 (0)	0 (0)	-
<b>Drug allergy- n (%)</b>	0 (0)	15 (8.2)	0 (0)	0 (0)	0.787
<b>Surgery- n (%)</b>	0 (0)	27 (14.7)	1 (14.3)	0 (0)	0.836
<b>Previous coexisting disease – n (%)</b>					
Hypertension	0 (0)	42 (22.8)	3 (42.9)	1 (33.3)	0.516
Diabetes	0 (0)	18 (9.8)	2 (28.6)	1 (33.3)	0.227
Heart disease	0 (0)	7 (3.8)	2 (28.6)	0 (0)	<b>0.021</b>
Pulmonary disease	0 (0)	2 (10.9)	0 (0)	1 (33.3)	< <b>0.001</b>
Liver disease	0 (0)	10 (5.4)	0 (0)	1 (33.3)	0.179
Kidney disease	0 (0)	3 (1.6)	0 (0)	0 (0)	0.978
<b>Origin- n (%)</b>					< <b>0.001</b>
Inland	0 (0)	166 (90.2)	6 (85.7)	3 (100)	-
Oversea	2 (100)	18 (9.8)	1 (14.3)	0 (0)	-
<b>P value is for Chi-square test or ANOVA.</b>					

Table 2  
Frequency of signs and symptoms according to COVID-19 phenotypes.

<b>Signs and symptoms</b>	<b>Mild (n =2)</b>	<b>Common (n =184)</b>	<b>Severe (n =7)</b>	<b>Critical (n =3)</b>	<b>Total (n = 196)</b>
Fever	0 (0)	155 (84.2%)	7 (100%)	3 (100%)	165 (84.2%)
Dry cough	2 (100%)	83 (45.1%)	3 (42.9%)	1 (33.3%)	89 (45.4%)
Expectoration	1 (50%)	30 (16.3%)	2 (28.6%)	1 (33.3%)	34 (17.3%)
Dyspnea	0 (0)	7 (3.8%)	1 (14.3%)	0 (0)	8 (4.1%)
Headache	0 (0)	11 (6%)	0 (0)	0 (0)	11 (5.6%)
Dizziness	0 (0)	5 (2.7%)	0 (0)	0 (0)	5 (2.6%)
Fatigue	0 (0)	7 (3.8%)	0 (0)	0 (0)	7 (3.6%)
Sore/Itchy/dry throat	0 (0)	12 (6.5%)	0 (0)	0 (0)	12 (6.1%)
Nasal congestion	0 (0)	7 (3.8%)	0 (0)	0 (0)	7 (3.5%)
Runny nose	0 (0)	7 (3.8%)	0 (0)	0 (0)	7 (3.5%)
Diarrhea	0 (0)	3 (1.6%)	0 (0)	0 (0)	3 (1.5%)
Myalgia	0 (0)	5 (2.7%)	0 (0)	0 (0)	5 (2.5%)
Chilly	0 (0)	8 (4.3%)	0 (0)	0 (0)	8 (4.1%)
Others symptoms	0 (0)	23 (12.5%)	0 (0)	1 (33.3%)	24 (12.2%)
<b>Initial therapy</b>					
Oxygen therapy	0 (0)	56 (30.4%)	7 (100%)	3 (100%)	66 (33.7%)
Prezcobix	0 (0)	14 (7.6%)	0 (0)	1 (33.3%)	15 (7.7%)
Arbidol	1 (50%)	40 (21.7%)	2 (28.6%)	2 (66.6%)	45 (22.9%)
Interferon	0 (0)	71 (38.6%)	2 (28.6%)	1 (33.3%)	74 (37.8%)
Lopinavir and Ritonavir Tablets	0 (0)	37 (20.1%)	2 (28.6%)	1 (33.3%)	40 (20.4%)
Meropenem	0 (0)	1 (0.5%)	0 (0)	1 (33.3%)	2 (1%)
Moxifloxacin	0 (0)	15 (8.1%)	0 (0)	1 (33.3%)	16 (8.2%)
Omeprazole	0 (0)	3 (1.6%)	0 (0)	2 (66.6%)	5 (1.6%)
Vitamin C	0 (0)	15 (8.2%)	1 (14.3%)	1 (33.3%)	17 (8.7%)
Hydroxychloroquine	0 (0)	7 (3.8%)	1 (14.3%)	0 (0)	8 (4.1%)
<b>Signs and symptoms were collected from the electronic medical records for the hospitalized patients</b>					



Signs and symptoms	Mild (n =2)	Common (n =184)	Severe (n =7)	Critical (n =3)	Total (n = 196)
ShuFengJieDuJiaoNang	0 (0)	10 (5.4%)	1 (14.3%)	0 (0)	11 (5.6%)
Others agents	0 (0)	27 (14.7%)	1 (14.3%)	2 (66.6%)	30 (15.3%)
<b>Signs and symptoms were collected from the electronic medical records for the hospitalized patients</b>					

## 3.2. Association of ACE2 SNPs and COVID-19

To find out further relationships between the 5 ACE2 polymorphisms and COVID-19, we used LightCycler®480 II system for *TaqMan @SNP Genotyping Assays* and LightCycler®480SW1.5.1 software for data analysis (Figure 1). Among the results of rs2048683 (T > G) genotyping (Fig. 1A, F), FAM fluorescent labeled probe targeted at base T and VIC fluorescent labeled probe targeted at base G. The prediction analysis of rs2048683 locus of 210 normal subjects (Fig. 1A) in peripheral blood was as follows: TT genotype 0 cases, TG genotype 0 cases, GG genotype 192 cases (red dots), and 18 cases were not classified into different genotypes (blue dots). The predicted analysis of the locus of 196 patients with COVID-19 (Fig. 1F) were as follows: TT genotype 1 case (blue dots), TG genotype 22 cases (green dots), GG genotype 168 cases (red dots), and 18 cases were not classified into different genotypes (purple dots). Among the results of rs4240157 (C > T) genotyping (Fig. 1B, G), FAM fluorescent labeled probe targeted at base T and VIC fluorescent labeled probe targeted at base C. The prediction analysis of rs4240157 locus of 210 normal subjects (Fig. 1B) in peripheral blood was as follows: CC genotype 7 cases (red dots), CT genotype 11 cases (green dots), TT genotype 181 cases (blue dots), and 11 cases were not classified into different genotypes (purple dots). The predicted analysis of the locus of 196 patients with COVID-19 (Fig. 1G) were as follows: CC genotype 7 case (red dots), CT genotype 4 cases (green dots), TT genotype 183 cases (blue dots), and 2 cases were not classified into different genotypes (purple dots). Among the results of rs4646142 (G > C) genotyping (Fig. 1C, H), FAM fluorescent labeled probe targeted at base G and VIC fluorescent labeled probe targeted at base C. The prediction analysis of rs4646142 locus of 210 normal subjects (Fig. 1C) in peripheral blood was as follows: CC genotype 81 cases (red dots), GC genotype 73 cases (green dots), GG genotype 53 cases (blue dots), and 3 cases were not classified into different genotypes (purple dots). The predicted analysis of the locus of 196 patients with COVID-19 (Fig. 1H) were as follows: CC genotype 76 case (red dots), GC genotype 51 cases (green dots), GG genotype 64 cases (blue dots), and 5 cases were not classified into different genotypes (purple dots). Among the results of rs6632677 (G > C) genotyping (Fig. 1D, I), FAM fluorescent labeled probe targeted at base G and VIC fluorescent labeled probe targeted at base C. The prediction analysis of rs6632677 locus of 210 normal subjects (Fig. 1D) in peripheral blood was as follows: CC genotype 37 cases (red dots), GC genotype 43 cases (green dots), GG genotype 122 cases (blue dots), and 3 cases were not classified into different genotypes (purple dots). The predicted analysis of the locus of 196 patients with COVID-19 (Fig. 1I) were as follows: CC genotype 12 case (red dots), GC genotype 15 cases (green dots), GG genotype 169 cases (blue dots), and 5 cases were not classified into different genotypes (purple dots). Among the results of rs2074192 (C > T) genotyping (Fig. 1E, J), FAM fluorescent labeled

probe targeted at base T and VIC fluorescent labeled probe targeted at base C. The prediction analysis of rs2074192 locus of 210 normal subjects (Fig. 1E) in peripheral blood was as follows: CC genotype 84 cases (red dots), CT genotype 69 cases (green dots), TT genotype 53 cases (blue dots), and 19 cases were not classified into different genotypes (purple dots). The predicted analysis of the locus of 196 patients with COVID-19 (Fig. 1J) were as follows: CC genotype 84 case (red dots), CT genotype 48 cases (green dots), TT genotype 59 cases (blue dots), and 5 cases were not classified into different genotypes (purple dots).

Next, we compared the genotype/allele frequencies between COVID-19 patients and normal controls, genotype/allele frequencies for ACE2 SNPs rs2074192, rs6632677, rs4646142, rs2048683, rs4240157 were listed (Table 3). Since genotype TT and GT of rs2024683 appeared zero in the normal group, data statistics could not be conducted, rs2024683 were eliminated. Base on Chi-square-test multiple inheritance models, the ACE2 SNP rs4240157 genotype frequency compare with control groups, without statistically significant differences. The dominant (OR = 0.24, 95% CI; 0.15-0.40,  $P < 0.001$ ), recessive (OR = 3.44, 95% CI; 1.71-6.58,  $P = 0.0002$ ), over-dominant (OR = 3.26, 95% CI; 1.79-6.28,  $P < 0.001$ ) model were all showed that rs6632677 is significantly associated with the prevalence of COVID-19, and the recessive (OR = 0.56, 95% CI; 0.35-0.90,  $P = 0.014$ ), over-dominant (OR = 1.69, 95% CI; 1.08-2.62,  $P = 0.020$ ) model were showed that rs2074192 was significantly related to COVID-19. For rs4646142, there were also significant differences between GC and GG genotypes (OR = 0.58, 95% CI; 0.34-0.96,  $P = 0.035$ ). These results suggested that ACE2 SNPs rs4646142, rs6632677 and rs2074192 were different between normal subjects and COVID-19 patients in our population.

Table 3  
Genotype and allele distribution of ACE2 SNPs.

SNP	Genotype/Allele frequency	Normal	COVID-19	Odd ratio (95% CI)	P value
rs4646142	GG	53 (25.6%)	64 (33.5%)	Ref	
	GC	73 (35.3%)	51 (26.7%)	0.58 (0.34-0.96)	<b>0.035</b>
	CC	81 (39.1%)	76 (39.8%)	0.78 (0.48-1.25)	0.303
	G	179 (43%)	179 (47%)	0.86 (0.66-1.14)	0.305
	C	235 (57%)	203 (53%)		
	CC vs GC+GG (dominant)			0.97 (0.65-1.45)	0.893
	CC+GC vs GG (recessive)			0.68 (0.45-1.06)	0.084
	CC+GG vs GC (over-dominant)			1.50 (0.98-2.27)	0.065
	rs20248683	TT	0 (0%)	1 (0.5%)	Ref
GT		0 (0%)	22 (11.5%)	-	-
GG		192 (100%)	168 (88%)	0 (0-7.92)	0.286
T		0 (0%)	24 (6%)	0 (0-0.13)	<b>&lt; 0.001</b>
G		384 (100%)	358 (94%)		
GG vs GT+TT (dominant)				Na (6.96-Na)	<b>&lt; 0.001</b>
GG+GT vs TT (recessive)				0 (0-8.95)	0.315
GT vs GG+TT (over-dominant)				0 (0-0.14)	<b>&lt; 0.001</b>
rs4240157		CC	7 (3.5%)	7 (3.6%)	Ref
	CT	11 (5.5%)	4 (2.1%)	0.36 (0.09-1.59)	0.196
	TT	181 (91%)	183 (94.3%)	1.01 (0.38-2.71)	0.984
	C	25 (6%)	18 (5%)	1.38 (0.76-2.50)	0.311
	T	373 (94%)	370 (95%)		
	TT vs CT+CC (dominant)			0.60 (0.27-1.28)	0.201
	TT+CT vs CC (recessive)			0.97 (0.36-2.61)	0.961

**P value is for Chi-square test, Abbreviation: OR, odds ratio, CI, Confidence Interval.**

SNP	Genotype/Allele frequency	Normal	COVID-19	Odd ratio (95% CI)	P value	
	CT vs TT+CC (over-dominant)			2.78 (0.95-8.06)	0.073	
rs6632677	GG	122 (60.4%)	169 (86.2%)	Ref		
	GC	43 (21.3%)	15 (7.7%)	0.25 (0.13-0.47)	< 0.001	
	CC	37 (18.3%)	12 (6.1%)	0.23 (0.12-0.46)	< 0.001	
	G	287 (71%)	353 (90%)	0.27 (0.18-0.40)	< 0.001	
	C	117 (29%)	39 (10%)			
	GG vs GC+CC (dominant)			0.24 (0.15-0.40)	< 0.001	
	GG+GC vs CC (recessive)			3.44 (1.71-6.58)	< 0.001	
	GC vs GG+CC (over-dominant)			3.26 (1.79-6.28)	< 0.001	
	rs2074192	CC	84 (44%)	84 (44%)	Ref	
		CT	69(36.1%)	48(25.1%)	0.70 (0.43-1.11)	0.135
TT		38 (19.9%)	59 (30.9%)	1.55 (0.95-2.60)	0.089	
C		237 (62%)	216 (57%)	1.26 (0.94-1.67)	0.122	
T		145 (38%)	166 (43%)			
CC vs CT+TT (dominant)				1 (0.67-1.50)	> 0.999	
CC+CT vs TT (recessive)				0.56 (0.35-0.90)	0.014	
CT vs CC+TT (over-dominant)				1.69 (1.08-2.62)	0.020	
P value is for Chi-square test, Abbreviation: OR, odds ratio, CI, Confidence Interval.						

### 3.3. Association of ACE2 SNPs and clinical indicators

C-reactive protein (CRP) is the main acute phase protein with a rapid and sharp increase in plasma concentration during infection and tissue damage, which can activate complement system and strengthen the phagocytosis of phagocytes. Acid glycoprotein (AGP) is the main acute response protein, which is increased during acute inflammation. Obviously, CRP and AGP play a vital protective role in the body's innate immune process. As shown in Fig. 2A.F, COVID-19 related ACE2 SNP rs4646142 was associated with increased CRP ( $P < 0.001$ ) and AGP ( $P < 0.001$ ), and rs6632677 may also be associated

with increased CRP ( $P = 0.052$ ). High-density lipoprotein (HDL) is a plasma lipoprotein that resists atherosclerosis and is a protective factor for coronary heart disease. Apolipoprotein A (ApoA) is also an important component of plasma HDL, which can effectively remove lipids from tissues and is considered an anti-atherosclerosis factor. As shown in Fig. 2C.D. ACE2 SNP rs4646142 were correlated with the abnormality of ApoA ( $P = 0.003$ ) and HDL ( $P = 0.009$ ). For Fig. 2B. ACE2 SNP rs4646142 was significantly associated with prealbumin (PAB) ( $P = 0.044$ ), and PAB is used as a sensitive nutritional protein indicator. For Fig. 2E. ACE2 SNP rs6632677 was further associated with HPT ( $P = 0.027$ ), and rs4646142 may also be associated with HPT although there is no significant difference ( $P = 0.083$ ). Dramatically, ACE2 SNP rs2074192 was not correlated with any clinical indicators.

## 4. Discussion

Notably different responses to SARS-CoV-2 infection among different populations raise the possibility that different SNPs profile might be responsible for the risk of acute inflammation, cardiovascular, hypertension, diabetes, and stroke in COVID-19 patients. In the present study, we identified in our population ACE2 SNPs rs4646142, rs6632677 and rs2074192, are associated with COVID-19 following SARS-CoV-2 infection. As far as we know, this is the first genetic study that has shown rs4646142, rs6632677 and rs2074192 may increase the odds of COVID-19 infection with previous coexisting disease in Chinese. Recent reports in Caucasian individuals determined the ACE2 rs2285666 variant was related to hypertension in elderly population (24). Taken together, the findings indicate a prominent role of ACE2 polymorphisms in the pathogenesis of COVID-19.

ACE2 is a transmembrane protein and the main entry point for some coronaviruses into cells, including SARS-CoV and SARS-CoV-2 (25). ACE2 may be related to a higher number of membrane-bound viral binding sites, which causing the vulnerability of carriers to infection. After the receptor binding domain (RBD) in the S1 domain of SARS-CoV and SARS-CoV-2 viral spike proteins bind to the extracellular region of ACE2, and spikes may be cleaved by TMPRSS2 on the cell surface, promoting the fusion of the outer membrane of the virus and the host cell membrane to allow the virus to enter the cytoplasm (26). Previous studies have reported that among patients with COVID-19 and coexisting hypertension, the mortality was lower through taking ACE inhibitors or angiotensin II receptor blockers (ARBs), when compared with COVID-19 patients with hypertension not taking such drugs (27). One study put forward a hypothesis on the role of Renin-Angiotensin-System (RAS) pathway genes including ACE2 (rs2285666, rs1978124, rs714205) in COVID-19 prognosis: inherited genetic predispositions can forecast the degree of severity of COVID-19 (28). Besides, the rs2285666 allele (T or A) was significantly positively correlated with lower infection and mortality among Indian population (29).

As can be seen from the baseline clinical characteristics (Table 1), the pathological degree of the COVID-19 is associated with previous coexisting disease, especially hypertension and diabetes. In addition, based on the literature search (<https://pubmed.ncbi.nlm.nih.gov/>), ACE2 SNPs are mainly related to human cardiovascular, hypertension, dyslipidemia, left myocardial hypertrophy, diabetes, stroke, retinopathy, etc (Table S1). Next, through the sequence of UCSC hg19 (exome) and the reference gene

Homo sapiens angiotensin I converting enzyme 2 (ACE2, NG\_012575.1:6110-46037) on chromosome X, 5 ACE2 SNPs (rs4646142 G > A or C, rs2048683 T > A or G, rs4240157 C > G or T, rs6632677 G > C, rs2074192 C > T) known to be associated with human cardiovascular diseases and diabetes were checked. Notably, research showed that SARS-CoV-2 is most closely associated with bat coronaviruses, 100% amino acid resemblance to bat SL-CoVZC45 in the E proteins and nsp7. That is, bats are a possible host of SARS-CoV-2 virus (30). Interestingly, rs4646142, rs2048683, rs4240157, rs6632677 and rs2074192 were completely consistent with bats ACE2 SNPs, while the SNPs in snakes were significantly different (Fig. S1), suggesting that these SNPs may be associated with the infection of SARS-CoV-2. We hypothesized that ACE2 variants rs2074192 and rs6632677 could modified the disease outcome.

In this study, we investigated possible associations between ACE2 polymorphisms and COVID-19 in our collected population. we found that ACE2 SNPs rs4646142, rs6632677, rs2074192 might correlated with the susceptibility of COVID-19 related cardiovascular risk and acute inflammatory infection. So why exactly do these SNPs impact susceptibility to COVID-19? We found that rs4646142, rs6632677 and rs2074192 were located in the intron region and could not encode amino acids. This may be related to the adjustment mode of the intron sequence. For instance, existing research shows that NS1 protein-RNA interactome, and NS1 primarily binds intronic sequences as a multifunctional virulence factor of the influenza a virus, thereby inhibiting cellular processes to accelerate viral gene expression (31). Human transformer 2 alpha homolog (huTRA2A) can inhibit mRNA splicing by binding to the intron silencer motif in the NS mRNA of the PR8/H1N1 virus (32). Some SNPs in tonicity-responsive enhancer binding protein (TonEBP) can impact transcription process as cis-expressed quantitative trait loci. Meanwhile, these SNPs are associated with increased risk of T2D, inflammation, hypertension, indicating that changes in TONEBP expression may be related to these phenotypes (33). In other words, these SNPs may be able to impact SARS-Cov2 infection process by binding to regulatory proteins or RNAs.

## 5. Conclusions

In conclusion, our study showed that the pathological degree of the COVID-19 may be associated with hypertension and diabetes. And, ACE2 gene variants were associated with the degree of severity in COVID-19 patients. Furthermore, ACE2 SNPs rs4646142 and rs6632677 may be an optimal genetic susceptibility marker for COVID-19 related cardiovascular complications, by evaluated the correlations between clinical indicators and SNPs. All in all, our findings supported that ACE2 SNPs rs4646142 and rs6632677 correlated with COVID-19 related cardiovascular risk, and it should be evaluated in large-scale studies.

## Abbreviations

ACE2

Angiotensin-converting enzyme 2

COVID-19

Coronavirus disease 2019

SNP  
single-nucleotide polymorphism  
hs-CRP  
high-sensitive C-reactive protein  
PAB  
prealbumin  
APOA  
apolipoprotein A  
HDL  
high-density lipoprotein  
AGP  
acid glycoprotein  
HPT  
haptoglobin  
SARS-CoV-2  
Severe acute respiratory syndrome coronavirus 2  
PPI  
protein-protein interaction  
gDNA  
Genomic DNA  
RBD  
receptor binding domain  
ARBs  
angiotensin II receptor blockers  
RAS  
Renin-Angiotensin-System  
huTRA2A  
Human transformer 2 alpha homolog  
TonEBP  
tonicity-responsive enhancer binding protein  
T2D  
Type 2 diabetes mellitus.

## **Declarations**

### **Ethics approval and consent to participate**

All procedures were reviewed and approved by the Ethics Committee of Shanghai Public Health Clinical Center, Fudan University, and have obtained written informed consents from all subjects.

### **Consent for publication**

All authors read, contributed to, and consent for publication.

### **Competing interests**

The authors declare no conflicts of interests.

### **Funding**

This research was supported by a grant from Science and Technology Commission of Shanghai (20Y11900700, 20411950200 and 20Z11900900), a grant from Special Research Fund of Youan Medical Alliance for the Liver and Infectious Diseases (LM202020), a grant (2018ZX10302103-003) from the National Special Research Program of China for Important Infectious Diseases and a grant from the National Natural Science Foundation of China (81672383).

### **Authors' contributions**

All authors read, contributed to, and approved the final manuscript.

### **Acknowledgments**

Thanks to Shanghai Public Health Clinical Center for providing samples.

### **Availability of data and material**

The datasets used or analysed during the current study are available from the corresponding author on reasonable request.

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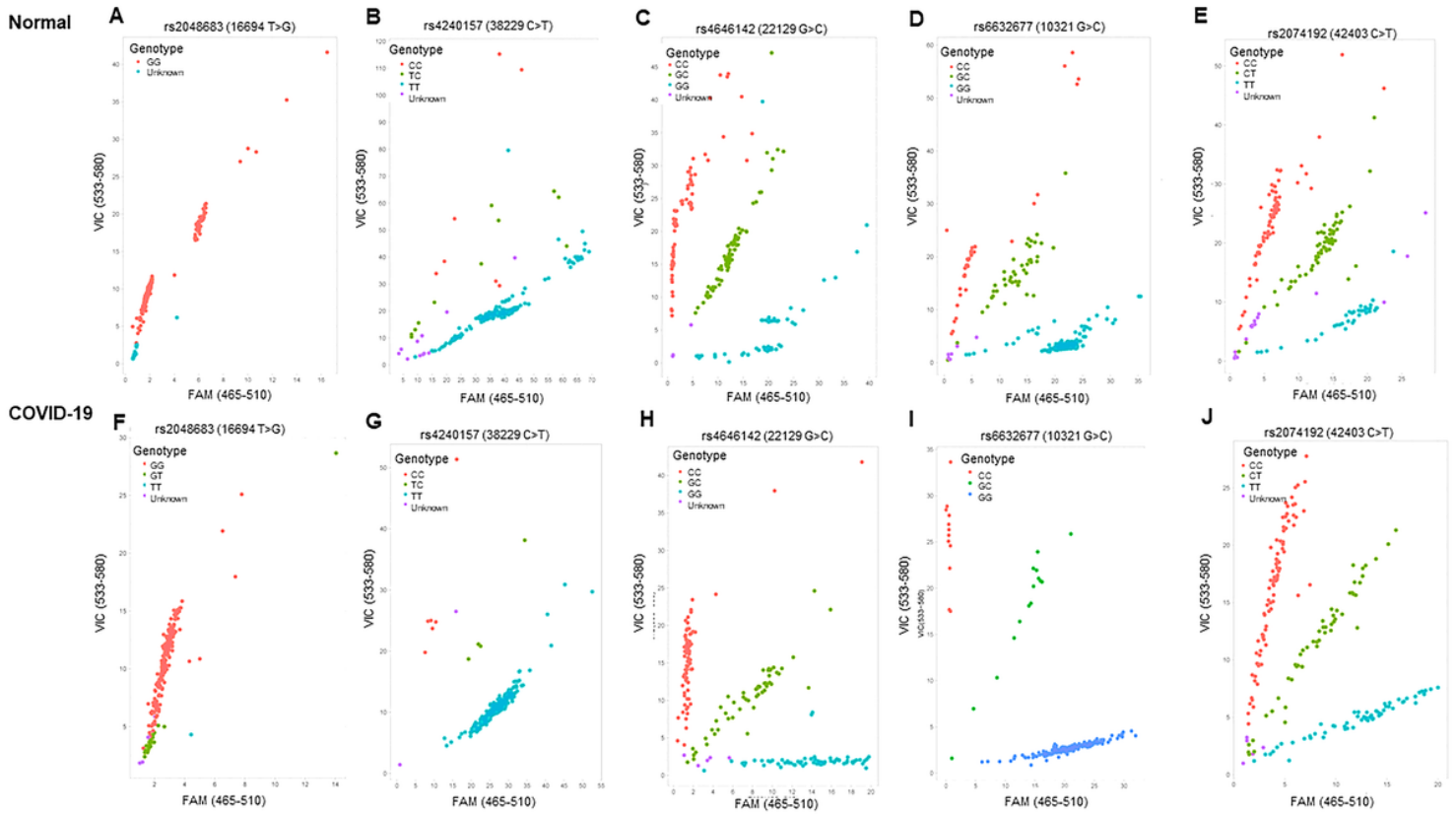
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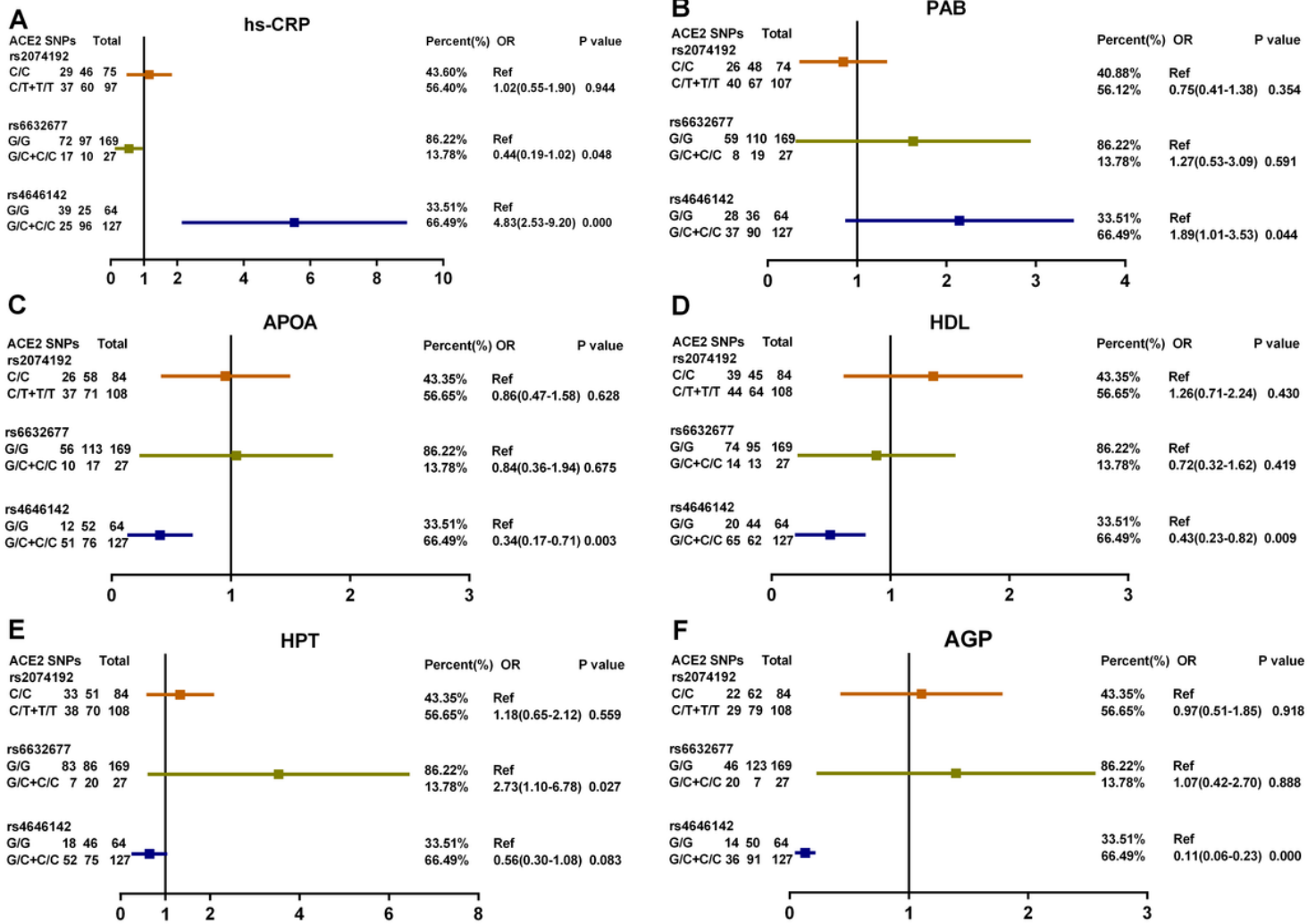
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## Figures



**Figure 1**

Fluorescence scatter diagrams of the ACE2 SNPs in normal controls (A-E) and COVID-19 patients (F-J). A, F) rs2048683; B, G) rs4240157; C, H) rs4646142; D, I) rs6632677; E, J) rs2074192.



**Figure 2**

Associated of diseases risk related ACE2 SNPs with clinical biochemical criterion in study participants. A) A high-sensitive C-reaction (hs-CRP); B) Prealbumin (PAB); C) Apolipoprotein A (APOA); D) High-density lipoprotein (HDL); E) Haptoglobin (HPT); F) Acid glycoprotein (AGP).

## Supplementary Files

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