Research paper

SARS-CoV-2 infections and COVID-19 mortalities strongly correlate with *ACE1* I/D genotype

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ABSTRACT

Severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) causes coronavirus disease 2019 (COVID-19). The relentless spread and pathogenicity of the virus have become a global public health emergency. One of the striking features of this pandemic is the pronounced impact on specific regions and ethnic groups. In particular, compared with East Asia, where the virus first emerged, SARS-CoV-2 has caused high rates of morbidity and mortality in Europe. This has not been experienced in past global viral infections, such as influenza, severe acute respiratory syndrome (SARS) and Middle East respiratory syndrome (MERS) and is unique to SARS-CoV-2. For this reason, we investigated the involvement of genetic factors associated with SARS-CoV-2 infection with a focus on angiotensin-converting enzyme (ACE)-related genes, because ACE2 is a receptor for SARS-CoV-2. We found that the *ACE1* II genotype frequency in a population was significantly negatively correlated with the number of SARS-CoV-2 cases. Similarly, the *ACE1* II genotype was negatively correlated with the number of deaths due to SARS-CoV-2 infection. These data suggest that the *ACE1* II genotype may influence the prevalence and clinical outcome of COVID-19 and serve as a predictive marker for COVID-19 risk and severity.

1. Introduction

Severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) has spread worldwide, and coronavirus disease 2019 (COVID-19) is now a pandemic with over 7.8 million infected people and over 430,000 deaths (as of June 15, 2020) (https://coronavirus.jhu.edu/map.html). Shortly after the first reported case in Wuhan, China, the virus spread rapidly to other Asian countries. SARS-CoV-2 has also spread to Central and Northern Europe and the Americas.

Central Europe has experienced a far greater number of cases and deaths from COVID-19 than East Asia, where the disease originally occurred (Zhu et al., 2020). Such population differences are unique to COVID-19 as they were not recognized in the severe acute respiratory syndrome (SARS) and the Middle East respiratory syndrome (MERS) that emerged in 2002 and 2012, respectively.

In addition to apparent socio-behavioral differences, European and East Asian people have differences that could facilitate SARS-CoV-2 infection including virological and immunological factors. In infectious diseases, such as COVID-19, the host genetic factors thought to define resistance or susceptibility to infection are also important. The angiotensin-converting enzyme (ACE) 2, a host cell receptor for SARS-CoV-2 (Hoffmann et al., 2020), has an analog called ACE1, and these

molecules, together with renin and angiotensin, constitute the renin angiotensin aldosterone system (RAAS) (Fountain and Lappin, 2019). RAAS is a general term for hormone systems related to regulation of blood pressure and extracellular volume. The human *ACE1* gene on chromosome 17 has an insertion (I) or deletion (D) of a 287 base pair (bp) Alu repeat sequence in intron 16 (Rieder et al., 1999). Therefore, in the I/D polymorphism, there are three different genotypes, II, ID and DD.

European and Asian people have continued a long-term migration eastward and westward across the vast Eurasian continent since the exodus of modern human ancestors from Africa about 200,000 years ago (Stringer and Galway-Witham, 2018) and during that time have achieved genetic variation. Therefore, in the current study, we investigated the genotypic differences in RAAS-related genes, *ACE1* and *ACE2*, to explain the ethnic difference in infection rate/mortality rates due to SARS-CoV-2 infection in European and Asian countries.

2. Materials and methods

2.1. Determination of ACE1 I/D genotypes

ACE1 insertion/deletion (I/D) genotypes were determined from high-coverage sequenced data of the phase 3 panel of the international 1000 Genomes Project (1000Genomes) and the Korean Personal Genome Project (KPGP) (doi: https://doi.org//10.1038/s41598-018-23837-x). The mapped read files in CRAM format of the phase 3 panel of the 1000 Genomes Project were retrieved from the European Nucleotide Archive. The raw read files in FASTQ format were retrieved from a File Transfer Protocol (FTP) server of KPGP. Sequence reads were aligned to GRCh38 reference sequences by BWA-mem (ver. 0.7.17-r1188) followed by the removal of duplicate reads by PicardTools (version 1.93). Single nucleotide variants (SNV) and short insertion/deletion (indel) were identified with GATK 4.1 according to the developer's protocol. SNV and indel discovery was conducted by HaplotypeCaller for each sample. Genotypes were determined using the GenotypeGVCFs program followed by filtration by Variant Quality Score Recalibration (VQSR). The genotype of *ACE1* polymorphism which is a 287 bp insertion of an ALU element at chr17:63488529 was determined by paraGRAPH v2.3 (https://doi.org/10.1186/s13059-019–1909-7).

Data on the geographical variation of the I/D polymorphism of the *ACE1* were also collected from published studies by Saab *et al* and others (Table 1), which include studies of 19 countries from Europe, the Middle East, South Asia and East Asia (Sweden, Denmark, United Kingdom, the Netherlands, Hungary, Belgium, Germany, France, Spain, Italy, Turkey, Lebanon, Kuwait, United Arab Emirates, India, China, South Korea, Taiwan and Japan) (Saab et al., 2007). Additionally, data on *ACE1* I/D genotypes from six countries (Portugal, Switzerland, Poland, Slovakia, Iran and Israel) were retrieved from the literature (Borzyszkowska et al., 2012; Freitas et al., 2008; Frishberg et al., 1998; Nikzamir et al., 2008; Siváková et al., 2009; Walder et al., 1998).

2.2. Single nucleotide polymorphism (SNP) and the allele frequency of the ACE2, CTSL and TMPRSS2

We obtained the allele frequency of the genes, *ACE2*, cathepsin L (*CTSL*) and transmembrane serine protease 2 (*TMPRSS2*) likely to be involved in the infection/pathogenicity of SARS-CoV-2 (Hoffmann et al., 2020; Ou et al., 2020), from the Genome Aggregation Database (gnomAD) (https://gnomad.broadinstitute.org/). gnomAD summarizes the variant information found in the whole genome sequences of about 140,000 people.

2.3. COVID-19 cases in each country

The number of COVID-19 cases and related fatalities were collected

from the Center for Systems Science and Engineering at Johns Hopkins University, as of May 23, 2020 (https://coronavirus.jhu.edu/map.html). The population of each country was obtained from the United Nations Population Division to calculate the number of cases and deaths per 1 million people.

ACE1 II genotype frequencies and the number of COVID-19 cases and deaths among European, Middle Eastern, South Asian and East Asian countries are summarized in Table 1.

3. Results

First, we investigated the correlation between *ACE1* I/D genotype and SARS-CoV-2 morbidity and mortality in Europe and Asia. Both the number of patients infected with SARS-CoV-2 (Fig. 1A) and the number of deaths from COVID-19 (Fig. 1B) were negatively correlated with the *ACE1* II genotype frequency, and the negative correlations were strong (R = − 0.847 and − 0.755, respectively). This suggests that the *ACE1* II genotype may affect the prevalence and clinical outcome of COVID-19. The European population has a lower *ACE1* II genotype frequency and a higher prevalence of and mortality due to COVID-19 than the Asian population. The *ACE1* II genotype frequency increases according to an eastward trend from European to Asian countries (Saab et al., 2007). A comparison of the median values obtained for cases/population and deaths/population of the European and Asian populations shows that the difference between deaths/population is greater than that between cases/population. This finding indicates that the European population has, in addition to a higher incidence of SARS-CoV-2 infection and associated disease, a higher probability of dying from the disease.

A similar analysis was performed, adding data points from countries in the Middle East and India collected from the literature (Table 1), and the same pattern as described above was observed (Fig. 2). Though weaker than the correlation observed without the Middle Eastern populations, the number of infected individuals still showed a fairly strong correlation with the *ACE1* II genotype (R = − 0.732) (Fig. 2A). Again, the comparison of the median values indicated that the European and Middle Eastern populations have a higher probability of acquiring SARS-CoV-2 infection compared with the Asian population. The correlation between the *ACE1* II genotype and the number of deaths was also weaker than that observed without Middle Eastern population but still demonstrated a moderate negative correlation (R = −0.452) (Fig. 2B).

Other genes involved in the pathogenicity of SARS-CoV-2 such as *ACE2*, *CTSL* and *TMPRSS2* were also studied; however, no significant correlation with COVID-19 prevalence or mortality was observed so far.

4. Discussion

Our results suggest that the *ACE1* I/D polymorphism may be one of the genetic markers for SARS-CoV-2 infectivity and pathogenicity. In addition to medical issues, a polymorphism in the *ACE1* , which is located in chromosome 17, is anthropologically very interesting. The geographical eastward migration of humans seems to coincide with a gradual increase in the frequency of the *ACE1* II genotype. Based on the Mantel test results, there is a significant correlation between geographical distance and genetic distance between populations (r = 0.478984, P < 0.0001) (Saab et al., 2007). It is therefore noteworthy that an increased frequency of the II genotype in the *ACE1* was inversely correlated with susceptibility to SARS-CoV-2 infection and consequent mortality. Therefore, mortality was not simply proportional to the number of infected/diseased cases, but appeared to be affected independently and additionally to the infected/diseased state. Under physiological conditions, the signal from angiotensin (Ang) II generated by ACE1 is related to pathological conditions such as vasoconstriction, inflammation and fibrosis, and ACE2-derived peptide, Ang 1–7, reverses the action of Ang II via the Mas receptor (Xiao et al., 2020).

Table 1

ACE1 II genotype frequency and the number of COVID-19 cases and deaths among European, Middle Eastern, South Asian and East Asian countries.

Country	Study authors	Year of publication	No. of subjects	*ACE1* II frequency (%)	#COVID-19 cases	case/pop (n/million)	deaths	death/pop (n/million)
Sweden	Kurland et al.	2001	59	27	33,188	3307	3,992	398
Denmark	Bladbjerg et al.	1999	199	23	11,487	1990	561	97
United Kingdom	Kehoe et al.	1999	386	23	258,504	3828	36,757	544
United Kingdom	Steeds et al.	2001	507	22	258,504	3828	36,757	544
United Kingdom	Narain et al.	2000	342	18	258,504	3828	36,757	544
Netherlands	Hosoi et al.	1996	61	20	45,265	2648	5,830	341
Hungary	Barkai et al.	2005	120	27	3,741	386	482	50
Belgium	Gu et al.	1994	109	19	56,810	4923	9,237	800
Germany	Ebert et al.	2005	145	23	179,986	2155	8,261	99
Germany	Filler et al.	2001	200	18	179,986	2155	8,261	99
France	Blanche et al.	2001	560	18	182,036	2795	28,218	433
France	Girerd et al.	1998	340	17	182,036	2795	28,218	433
Spain	Alvarez et al.	1999	400	15	235,290	5034	28,678	614
Spain	Coll et al.	2003	133	15	235,290	5034	28,678	614
Italy	Di Pasquale et al.	2005	684	18	229,327	3787	32,735	541
Italy	Panza et al.	2002	252	13	229,327	3787	32,735	541
Portugal	Freitas et al.	2008	510	16	36,690	3569	1,517	148
Switzerland	Walder et al.	1998	199	25	31,117	3631	1,938	226
Poland	Borzyszkowska et al.	2012	632	29	29,392	774	1,247	33
Slovakia	Siváková et al.	2009	209	25	1,548	284	28	5
Iran	Nikzamiret et al.	2008	51	16	187,427	2291	8,837	108
Israel	Frishberg et al.	1998	216	10	19,121	2152	302	34
Turkey	Tanriverdi et al.	2005	102	23	150,388	1834	4,182	51
Turkey	Serdaroglu et al.	2005	287	23	150,388	1834	4,182	51
Turkey	Bedir et al.	1999	143	23	150,388	1834	4,182	51
Lebanon	Saab et al.	2007	570	7	1089	159	27	4
Kuwait	Al-Eisa et al.	2001	48	2	19,858	4800	145	35
United Arab Emirates	Saeed et al.	2005	130	6	27,198	2824	231	24
India	Patil et al.	2005	300	26	124,476	92	4,059	3
China	Thomas et al.	2001	119	33	84,084	59	4,638	3
China	Ohishi et al.	1994	175	37	84,084	59	4,638	3
China	Young et al.	1998	183	39	84,084	59	4,638	3
China	Iwai et al.	1994	122	41	84,084	59	4,638	3
China	Yan et al.	2005	352	41	84,084	59	4,638	3
Korea, South	Ryu et al.	2002	167	34	11,190	218	266	5
Korea, South	Um et al.	2003	613	37	11,190	218	266	5
Taiwan	Lee et al.	2002	750	47	441	19	7	0
Japan	Katoh et al.	2005	270	41	16,536	130	808	6
Japan	Odawara et al.	1997	248	42	16,536	130	808	6
Japan	Mannami et al.	2001	3657	43	16,536	130	808	6
Japan	Maguchi et al.	1996	84	48	16,536	130	808	6
Japan	Ishigami et al.	1995	87	51	16,536	130	808	6
Japan	1000G JPT	–	104	33	16,536	130	808	6
Korea, South	KPGP	–	88	41	11,190	218	266	5

Information of *ACE1* II genotype frequencies in each country were found in the references listed in the left half of the table, while those on the number of COVID-19 cases and deaths were collected from the Center for Systems Science and Engineering at Johns Hopkins University (https://coronavirus.jhu.edu/map.html) (as of May 23, 2020) and listed in the right half of the table, respectively.
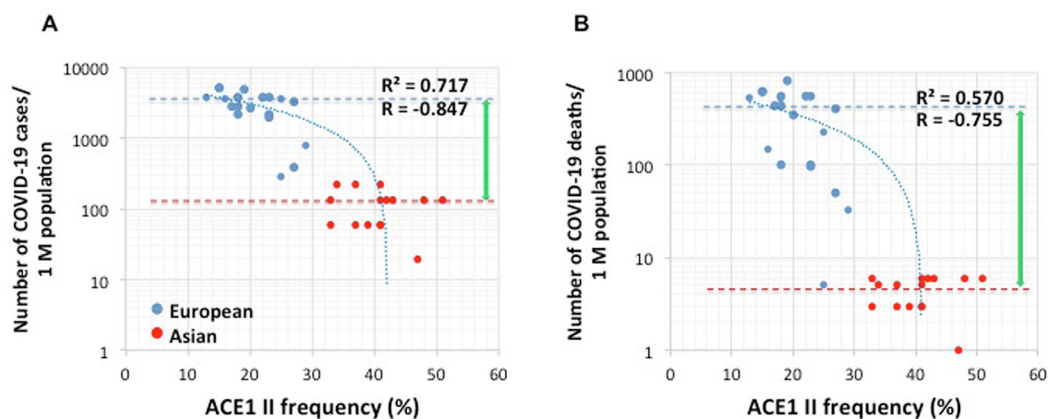

Fig. 1. Correlation between COVID-19 prevalence and *ACE1* II allele frequency (%) in Europe and Asia: (A) the number of COVID-19 cases/ million populations, $R^2 = 0.717$, $R = -0.847$, (B) the number of COVID-19-related deaths/ million population, $R^2 = 0.570$, $R = -0.755$. The dashed lines indicate the median values.
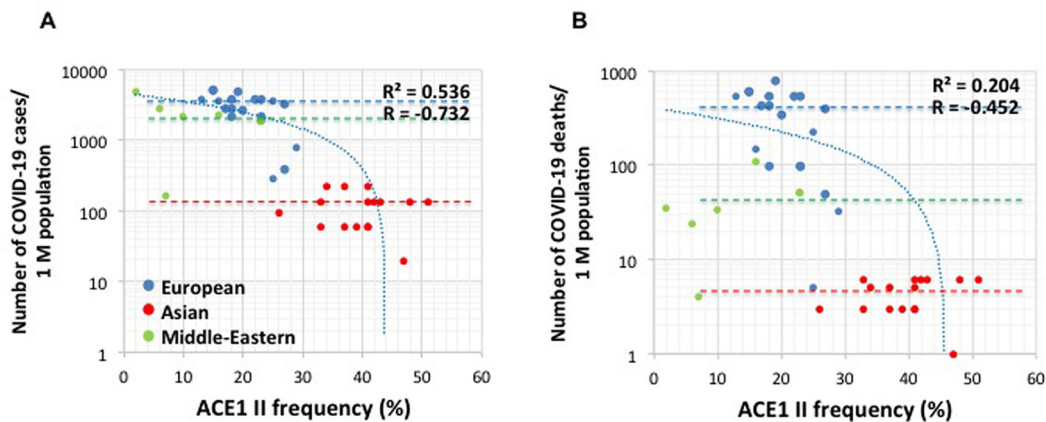
Fig. 2. Correlation between COVID-19 prevalence and *ACE1* II allele frequency (%) in Europe, the Middle East, India and East Asia: (A) the number of COVID-19 cases/ million populations, $R^2 = 0.536$, R = - 0.732, (B) the number of COVID-19-related deaths/ million population, $R^2 = 0.204$, R = - 0.452. The dashed lines indicate the median values.

Therefore, the balance between ACE1 and ACE2 is considered to be very important for maintaining homeostasis in the body (Chappel and Ferrario, 2006; Gemmati et al., 2020).

It has been reported that serum levels of ACE1 are significantly higher in those with the DD genotype compared with those with either the ID or II genotypes (Rigat et al., 1990). Moreover, because ACE2 is a receptor for SARS-COV-2 (Hoffmann et al., 2020), viral infection may lead to the suppression of ACE2 function and cause ACE1/ACE2 imbalance responsible for RAAS over-activation and pulmonary shutdown (Gemmati et al., 2020). This can further reduce the effects of ACE2, which counteract the pathophysiological effects of Ang II produced by ACE1, and may worsen the pathology. In patients with the D allele, especially those with the DD genotype, the risk of morbidity and mortality from acute respiratory distress syndrome (ARDS) (Marshall et al., 2002; Adamzik et al., 2007) and certain heart, lung and inflammatory conditions (Gard, 2010) is reported to be higher. The ACE1/ACE2 imbalance predicts that COVID-19 patients with the D allele of *ACE1*, especially the DD genotype will have a higher severity of disease as seen in SARS patients with an *ACE1* DD genotype (Itoyama et al., 2004). However, one report found that the *ACE1* I/D polymorphism is not directly related to susceptibility to SARS-CoV infection nor the development of SARS (Chan et al., 2005).

ACE1 I/D polymorphism is also thought to be associated with the cough reflex (Morimoto et al., 2002). It is known that aspiration due to a decrease in cough with aging is the main cause of pneumonia in the elderly. Morimoto et al. reported that the *ACE1* D allele contributes to the risk of pneumonia in elderly Japanese individuals (Morimoto et al., 2002). They propose a model in which the high expression of ACE1 in the *ACE1* DD genotype reduces substance P and increases the risk of pneumonia due to a reduced cough reflex. Additionally, a Chinese group conducted a *meta*-analysis of 12 studies on pneumonia and *ACE1* including studies from Japan and the Netherlands, and concluded that the *ACE1* I/D polymorphism and pneumonia risk are significantly associated (Nie et al., 2014). These studies, which were conducted during the pre-COVID-19 era, are in relative agreement with the observation of many fewer cases and deaths due to the COVID-19 in Asian populations with a high frequency of *ACE1* II genotypes.

We discovered that a gene (*ACE1*) and its genotype (I/D) seem to provide a plausible explanation for why some ethnic groups, especially Europeans populations, have been more heavily affected by SARS-CoV-2 than Asians populations. We believe that this is a very unique and important issue in infectious diseases. Such an ethnic difference was never experienced in the past, even in the influenza pandemic of 1918 that caused high rates of mortality or with SARS and MERS, which in many respects are thought to be very similar to COVID-19. In this analysis, we have shown for the first time that the prevalence of the D

allele in the *ACE1* gene is integrally involved in susceptibility to SARS-CoV-2 infection and the exacerbation of COVID-19 symptoms such as pneumonia. The association of the I/D polymorphism in the *ACE1* with susceptibility and outcome of ARDS has been reported (Marshall et al., 2002). This finding is closely linked to the current results observed in patients infected with SARS-CoV-2.

Middle Eastern populations, especially, those from Lebanon, are believed to be ancestral with regard to the *ACE* polymorphism and have a relatively low frequency of the insertion allele (Saab et al., 2007). In our analysis of data from the literature, which included Middle Eastern countries, the association between SARS-CoV-2 infections/mortalities and *ACE1* I/D genotype was clearly reduced (Fig. 2). Indeed, Delanghe et al. recently showed a correlation between increasing D alleles and decreasing COVID-19 morbidity/mortality from an analysis of 33 countries in Europe, North Africa and the Middle East (Delanghe et al., 2020). This is contrary to our observation. In North Africa and the Middle East, the onset of SARS-CoV-2 was clearly delayed compared with Asia and Europe. However, it is reported that COVID-19 now poses a formidable threat to fragile countries in the Middle East (https://coronavirus.jhu.edu/map.html). This suggests that more complex factors are involved in the Middle East. This view may be further supported from our observations that people in Europe and the Middle East have a higher probability of acquiring SARS-CoV-2 infection compared with the Asian people, whereas the correlation between *ACE1* II genotype and COVID-19 mortality was weakened when data from the Middle East was added (Figs. 1 and 2). Will the Middle East continue to have the unique situation of high numbers of infected people and low deaths? Therefore, the relationship between SARS-CoV-2 and genetic factors in the Middle East is an important focus for future investigation.

In the Americas, although the onset of infection was slightly delayed compared with Europe, the number of cases of SARS-CoV-2 infection (at the time of this communication) is rapidly increasing. The situation in the United States is the most serious with the number of people infected or deceased now as large as nearly 30% of the infected/deceased worldwide. Similarly, the number of new cases is rapidly increasing in Latin America and the Caribbean, including Brazil, Peru, Chile and Mexico. Populations in these countries were generally formed by immigrants from around the world in addition to native Americans originally coming from Asia. In this sense, analytical research focusing on ethnic differences is important. The advantage of research in these countries is that it makes it possible to investigate the racial differences that COVID-19 infection causes in very similar environments. It is anticipated that, in the near future, the accumulated data from the United States and other countries will provide a more accurate understanding of the role of *ACE1* I/D polymorphism in SARS-CoV-2 infection and COVID-19-related morbidity/mortality.

Regarding the genetic predisposition of COVID-19, other genes may be involved, and a combination of multiple genes may affect the severity of infection. Although SNPs occur with high frequency in *ACE2*, *CTSL* and *TMPRSS2*, and over 1% of differences among populations are present in these genes, the significance of these SNPs in SARS-CoV-2 infection/pathogenicity is not clear. Human leukocyte antigens (HLA) gene polymorphisms are associated with various diseases such as autoimmune diseases and infectious diseases. Because HLA is a protein of the immune system responsible for antigen presentation, HLA has been attracting attention in relation to disease susceptibility. However, there is no correlation between the global distribution of HLA alleles frequency and allele ability to bind SARS-CoV-2 peptides (Barquera et al., 2020; Nguyen et al., 2020). Furthermore, there is as yet no report of HLA involvement in SARS-CoV-2 infection and COVID-19 pathology.

5. Conclusions

There was a strong negative correlation between the number of SARS-CoV-2 cases and the number of deaths due to viral infection, which decreased with increasing *ACE1* II genotype frequency. This suggests that the *ACE1* I/D genotype may be involved in various pathological conditions caused by SARS-CoV-2 infection such as pneumonia, disseminated intravascular coagulation and thrombosis, ischemic stroke, renal injury and immune response such as cytokine storm. Therefore, an urgent task is to assess the clinical outcome of SARS-CoV-2 infection in DD, ID, and II carriers and study the exact role of *ACE1*. Further studies on COVID-19 and *ACE1* polymorphisms are expected to promote the prediction of high-risk groups and the treatment of COVID-19 patients.

CRediT authorship contribution statement

Naoki Yamamoto: Writing - original draft, Investigation, Supervision. **Yasuo Ariumi:** Writing - original draft, Investigation. **Nao Nishida:** Formal analysis, Software. **Rain Yamamoto:** Formal analysis, Software. **Georg Bauer:** Investigation, Conceptualization. **Takashi Gojobori:** Investigation, Conceptualization. **Kunitada Shimotohno:** Investigation, Conceptualization, Supervision. **Masashi Mizokami:** Supervision.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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