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Zoonotic Disease: A Highly Conservedbinding Region of Angiotensin-Converting Enzyme2as a Receptor for Severe Acute Respiratory Syndromecoronavirus 2 (SARS-Cov-2) between Humans and Mammals

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ABSTRACT

A case of the new coronavirus severe acute respiratory syndromecoronavirus 2(SARS-CoV-2) infection transmitting from a human to a domestic cat was reported on March 27, 2020 in Belgium. Cases of SARS-CoV-2 infection being transmitted from owners to their dogs have also been reported. For the first time, however, a case of SARS-CoV-2 transmission from a human to a domestic cat has been confirmed. A tiger kept at a zoo in New York was also reportedly infected with SARS-CoV-2; it is believed that the virus was transmitted to the tiger from infected caretakers. Therefore, we examined the homology of the angiotensin-converting enzyme2 (ACE2) molecule, a receptor for the spike glycoprotein on the virionsurface of SARS-CoV-2, between humans, dogs, cats, and other mammals. We found that the binding region of theACE2 molecule with the SARS-CoV-2 spike glycoprotein has high homology between humans, dogs, cats, tigers, and other mammals. Although the transmission of human coronavirus to petsor animals is rare, SARS-CoV-2 transmission from animals or pets to humans have not yet been demonstrated. The Belgian Health Service considers zoonotic infection as a special case. However, the Belgian Health Service has suggested individuals with SARS-CoV-2 to also avoid contact with pets or other animals.

KEYWORD: *ACE2, Binding region, COVID-19, Severe acute respiratory syndrome coronavirus 2*

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INTRODUCTION

A novel human coronavirus now known as severe acute respiratory syndromecoronavirus 2 (SARS-CoV-2) emerged in Wuhan, China in late 2019 and is now causing a worldwide pandemic [1].Thegenome of SARS-CoV-2 shares approximately 80% of its identity with that of SARS-CoV and is approximately 96% identical to the bat coronavirus RaTG13 [2].In the case of SARS-CoV, the spike glycoprotein on the virionsurface mediates receptor recognition and membrane fusion [3,4,5]. During viral infection, the trimeric spike glycoprotein is cleaved into the S1 and S2 subunits and the S1 subunits are released in the transition to the post fusion conformation[4,5,6,7].The S1 subunit contains the receptor binding domain, which directly binds to the binding region located in the peptidase domain of the angiotensin-converting enzyme 2 (ACE2), where as the S2subunit is responsible for membrane fusion [5,6] (Fig. 1).

According to a report by the Belgian Health Service, a veterinary team in Liege confirmed cases of transmission from humans to domestic cats. In Liege, located in eastern Belgium, SARS-CoV-2 infection was observed in a domestic cat owned by an individual infected with SARS-CoV-2. One week after a woman showed symptoms of coronavirus disease 2019 (COVID-19), the cat experienced symptoms of diarrhea, vomiting, and dyspnea. Following testing for infectious diseases, SARS-CoV-2 was detected in the cat's feces. At present, the cat's condition is improving. A report from the Bronx Zoo in New York indicated that four tigers and three African lions were coughing and their appetite was decreasing, but all are recovering. Because large animals require general anesthesia, only one of the tigers was tested for SARS-CoV-2 infection. The test revealed that the 4-year-old female tiger was infected with SARS-CoV-2.

The route of transmission of SARS-CoV-2 from human owners or caretakers to dogs, cats, and other mammals has not yet been determined. Coronaviruses can infect humans and many animal species, including swine, cattle, horses, camels, cats, dogs, rodents, birds, bats, rabbits, ferrets, mink, pangolins, snakes, and others[7,8]. Results from a recent analysis suggest that SARS-CoV-2ismost similar to bat coronaviruses in terms of genetic information, and is most similar to snake coronaviruses in terms of cod on usage bias [9]. A Chinese research group has found genomic and evolutionary evidence of the occurrence of a SARS-CoV-2-like coronavirus (namedPangolin-CoV) in dead Malayan pangolins. Pangolin-CoV is 91.02% identical to SARS-CoV-2 and 90.55% identical to the bat coronavirusRaTG13 at the whole-viral-genome level [10].

Therefore, we examined the homology of the ACE2 molecule, which is reported as a cellular receptor for the spike glycoprotein on the virionsurface of SARS-CoV-2, between humans, dogs, cats, tigers, and other mammals. We found that the binding region of the ACE2 molecule with the spike glycoprotein of SARS-CoV has high homology between humans, dogs, cats, tigers, and other mammals.

Investigators in China reported the results of an open-label, randomized clinical trial of lopinavir–ritonavir for the treatment of COVID-19 in 199 infected adult patients. There were no differences in the primary end point or time to clinical improvement[11].In hospitalized adult patients with severe COVID-19, no benefit was observed with lopinavir–ritonavir treatment beyond standard care. Whether combining lopinavir–ritonavir with other antiviral agents, as has been done in SARSand is being studied for Middle East Respiratory Syndrome-CoV, might enhance antiviral effects and improve clinical outcomes remains to be determined[12].Our examinations will provide information to support precise vaccine design and the discovery of antiviral therapeutics, accelerating medical countermeasure development.

METHODS

Phylogenetic Analysis and Annotation

Reference genomes and amino acids of human, dog, cat, tiger, bat, pangolin, and snake ACE2s were obtained from the National Center for Biotechnology Information (NCBI) Orthologs of the National Library of Medicine. Amino acid homological analysis was performed using Align Sequences Protein BLAST (algorithm protein–protein BLAST) with the protein accession numbers of ACE2s listed in the NCBI Reference Sequence Database in order to determine the whole amino acid homology of ACE2 between humans and other animals. Phylogenetic analyses of the complete protein and major coding regions were performed with RAxML software (version 8.2.9) with 1000 bootstrap replicates using the general time reversible nucleotide substitution model. Details of the protein accession numbers of ACE2s are available in the supplementary materials.

Amino Acid Homology Analysis of the Binding Region of ACE2 for Interaction with SARS-CoV-2 Spike Glycoprotein between Humans and Other Animals

The binding region for interaction with the SARS-CoV-2 spike glycoprotein (82.aa-**MYP**-84.aa, 353.**aa-KGDFR**-357.aa)of the verified genome amino acid sequences of human ACE2 was predicted using the NCBI protein database and Geneious software (version 11.1.5; Auckland, New Zealand), and was annotated using the NCBI Conserved Domain Database. Amino acid homological analysis was performed using Align Sequences Protein BLAST (algorithm protein–protein BLAST) with the protein accession numbers of human and individual animal ACE2s listed in the NCBI Reference Sequence Database. Details of the protein accession numbers of ACE2s are available in the supplementary materials.

RESULTS

The outbreak of COVID-19 caused by the SARS-CoV-2 virus has now become a pandemic, but there is currently little understanding of zoonotic transmission and the antigeni city of the virus. We therefore examined the homology of the whole ACE2 molecule, which is reported as a cellular receptor for the spike glycoprotein on the virionsurface of SARS-CoV-2, between humans, dogs, cats, and other mammals. Our findings show that the ACE2 molecule demonstrated 79% to 92% homology between humans and dogs, 91% to 92% homology between humans and cats, and 92% homology between humans and tigers (Fig. 1).The ACE2 molecule showed 80% to 89% homology between humans and bats, 91% homology between humans and pangolins, and 74% to 75% homology between humans and snakes (Fig. 2).

In addition, we examined the homology of the five amino acids residues KGDFR, located in the binding region of the ACE2 molecule, which directly recognize and bind the spike glycoprotein on the virionsurface of SARS-CoV-2 between humans, dogs, cats, and other mammals. Our findings show that these five amino acid residues have 100% homology among humans, dogs, cats, tigers, and bats, 80% homology between humans and pangolins, and 60% homology between humans and snakes(Fig. 3).These results suggest that SARS-CoV-2 may transmit from humans to dogs, cats, and tigers.

DISCUSSION

Thus far, 17 dogs and 8 cats that had contact with individuals infected with SARS-CoV-2 have been tested for infection in Hong Kong. Test results indicated that only 2 of the dogs were positive for the SARS-CoV-2 infection. In these cases from Hong Kong, the dogs infected with SARS-CoV-2 did not show COVID-19 symptoms. On the other hand, according to a report by the Belgian Food Safety Agency, symptoms of COVID-19, including respiratory and digestive symptoms, were observed in domestic cats infected with SARS-CoV-2.Because the expression of ACE2 is found in many organs, such as human lung, liver, and small intestine, it is possible that expression of ACE2 may contribute to the development of digestive diseases. According to a report from the Bronx Zoo in New York, a 4-year-old female tiger with a cough was found to be infected with SARS-CoV-2.

Coronavirus can infect humans and many different animal species, including swine, cattle, horses, camels, cats, dogs, pangolins, rodents, birds, bats, rabbits, ferrets, mink, snakes, and other animals[7,8,13,14]. Results from a recent analysis suggest that SARS-CoV-2is most similar to bat coronaviruses in terms of genetic information and is most similar to snake coronaviruses in terms of codon usage bias [9].

Jason McLellan's team solved the structure of the SARS-CoV-2spikeglycoprotein using cryoelectron microscopy[1]. Biophysical assays demonstrated that the spike protein of SARS-CoV-2 binds to their common host cell receptor at least 10 times more tightly than the corresponding spike protein of SARS-CoV[15].In this report, the results showed high homology of five amino acids in the binding region of ACE2,suggesting that SARS-CoV-2 may transmit from humans to dogs and cats.

The route of transmission of SARS-CoV-2 from humans to dogs, cats, and tigers has not been determined by the current examinations. Aerosol and fomite transmission of SARS-CoV-2 are plausible because the virus can remain viable and infectious in aerosols for hours and on surfaces up to days, depending on the inoculum shed. These findings echo those found for SARS-CoV, in which these forms of transmission were associated with nosocomial spread and super-spreading events, and they provide information for pandemic mitigation efforts [15,16].SARS-CoV-2 can be detected in animal feces. Therefore, based on our results, in addition to the bats already reported, stray cats and dogs may also become carriers of SARS-CoV-2 and could be vectors for humans.

Investigators in China reported the results of an open-label, randomized clinical trial of lopinavir–ritonavir for the treatment of COVID-19 in 199 infected adult patients. There was no difference in the primary end point or time to clinical improvement [11,12]. One concern is whether people and animals develop durable immunity to SARS-CoV-2, which is crucial given that vaccines try to mimic a natural infection. Finally, pandemics will generate simultaneous demand for vaccines around the world. Clinical and serological studies will be needed to confirm which populations remain at highest risk when vaccines are available. These could then form the basis for establishing a fair vaccine-allocation system globally [19,20,21]. A group of seven countries has already called for such a global system, the planning of which must start while vaccine development proceeds.

CONCLUSION

Recent reports have demonstrated that the results of an open-label, randomized clinical trial of lopinavir–ritonavir for the clinical treatment of COVID-19 in 199 infected adult patients showed no differences in the primary end point or time to clinical improvement. In hospitalized adult patients with severe COVID-19, no benefit was observed with lopinavir–ritonavir treatment beyond standard care. The need to rapidly develop a vaccine against SARS-CoV-2 comes at a time of rapid expansion in basic scientific understanding, including in areas such as genomics and structural biology that support this new era in novel vaccine development. Thus far, there are no specific therapeutic agents for coronavirus infections.

The Belgian Health Service reports that there is no medical evidence of SARS-CoV-2 transmission from pets to humans or other pets. The risk of SARS-CoV-2 transmission from pets to humans is much lower than in cases of SARS-CoV-2 transmission due to human contact. However, to prevent cross-species transmission of SARS-CoV-2 from humans to pets, especially if the owner may be infected with SARS-CoV-2, pet owners should avoid close contact with their pets and should refrain from allowing pets to lick their faces. Our research will support precise vaccine design and the discovery of antiviral therapeutics, accelerating the development of medical countermeasures.

Footnote

ACE2-angiotensin-converting enzyme 2

The protein encoded by this gene belongs to the angiotensin-converting enzyme family of dipeptidyl carboxypeptidases and has considerable homology with thehuman angiotensinconverting enzyme1. This secreted protein catalyzes the cleavage of angiotensin I into angiotensin, and angiotensin II into the vasodilator angiotensin. The organ- and cell-specific expression of this gene suggests that it may play a role in the regulation of cardiovascular and renal functions, as well as fertility. In addition, the encoded protein is a functional receptor for the spike glycoprotein of the human coronaviruses SARS and HCoV-NL63. [Provided by RefSeq, Jul 2008: https://www.ncbi.nlm.nih.gov/gene/59272/ortholog/?scope=7776].

Abbreviations

ACE2, angiotensin-converting enzyme2; COVID-19, coronavirus disease 2019; NCBI, National Center for Biotechnology Information; SARS-CoV-2,severe acute respiratory syndrome coronavirus 2

Data Sharing

Data are available on various websites and have been made publicly available (more information can be found in the first paragraph of the Results section).

Disclosure

The authors declare no potential conflicts of interest. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

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Author Contributions

T.H. performed most of the experiments and coordinated the project; T.H. and M.M. conceived the study and wrote the manuscript. N.Y. and I.K. gave information on clinical medicine and oversaw the entire study.

Transparency document

The transparency document associated with this article can be found in the online version at http://

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Hayashi *et al*. Figure 1

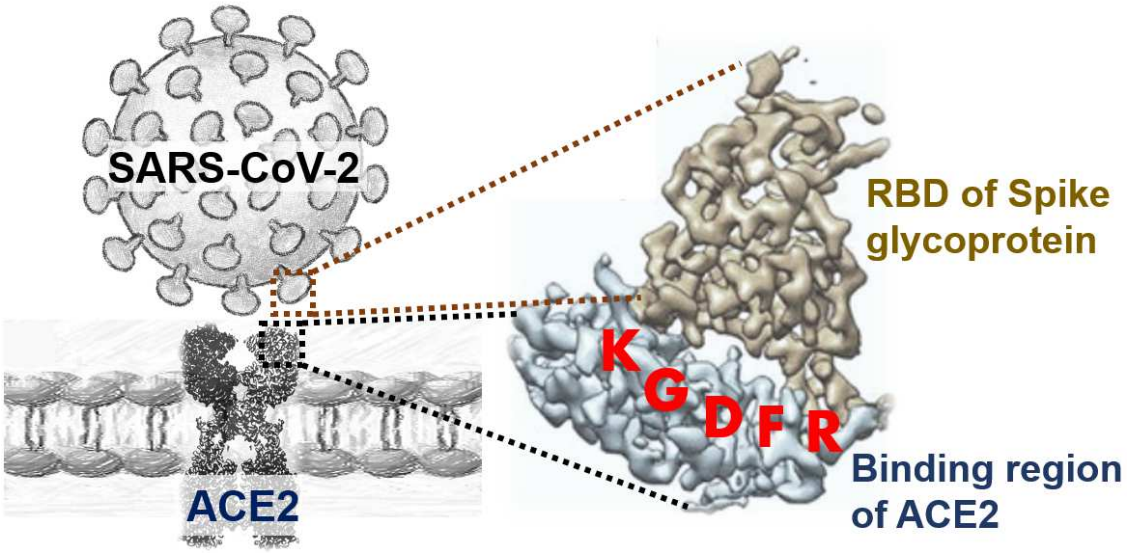

Figure 1 Interaction between SARS-CoV-2 and the Renin-Angiotensin-Aldosterone System

Shown is the initial entry of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) into cells, primarily type II pneumocytes, after binding to its functional receptor, angiotensin-converting enzyme 2 (ACE2).Receptor binding domain (RBD) of spike glycoprotein of SARS-CoV-2 directly recognizes and associates the binding region of angiotensin converting enzyme 2 (ACE2). The inset shows the focused refined map of 5 amino acids, KGDFR located in binding region of ACE2 (Structure summary MMDB ID 185055). Part of figure is adapted from Wrapp D. *et al.* 367(6483)(2020) 1260-1263.

Hayashi *et al.* Figure 2

Global Alignment » results for RID-8B0TUUHX114 Human ACE2 vs Mammals ACE2

Species			Protein ID	Homology
Human	[Homo sapiens]	NCBI Reference Sequence:	NP_001358344.1	
Dog#1	[Canis lupus familiaris]	NCBI Reference Sequence:	NP_001158732.1	91.0%
Dog#2	[Canis lupus familiaris]	NCBI Reference Sequence:	XP_005641049.1	92.0%
Dog#3	[Canis lupus familiaris]	NCBI Reference Sequence:	XP_013966804.1	92.0%
Dog#4	[Canis lupus familiaris]	NCBI Reference Sequence:	XP_022271214.1	79.0%
Cat#1	[Felis catus]	NCBI Reference Sequence:	XP_023104564.1	91.0%
Cat#2	[Felis catus]	NCBI Reference Sequence:	NP_001034545.1	92.0%
Tiger#1	[Panthera tigris altaica]	NCBI Reference Sequence:	XP_007090142.1	92.0%
Bat#1	[Myotis brandtii]	NCBI Reference Sequence:	XP_014399780.1	88.0%
Bat#2	[Myotis brandtii]	NCBI Reference Sequence:	XP_014399781.1	88.0%
Bat#3	[Myotis brandtii]	NCBI Reference Sequence:	XP_014399782.1	89.0%
Bat#4	[Myotis brandtii]	NCBI Reference Sequence:	XP_014399783.1	80.0%
Bat#5	[Desmodus rotundus]	NCBI Reference Sequence:	XP_024425698.1	88.0%
Bat#6	[Desmodus rotundus]	NCBI Reference Sequence:	XP_024425699.1	81.0%
Bat#7	[Eptesicus fuscus]	NCBI Reference Sequence:	XP_008153150.1	88.0%
Bat#8	[Eptesicus fuscus]	NCBI Reference Sequence:	XP_027986092.1	88.0%
Bat#9	[Myotis lucifugus]	NCBI Reference Sequence:	XP_023609437.1	88.0%
Bat#10	[Myotis lucifugus]	NCBI Reference Sequence:	XP_023609438.1	88.0%
Bat#11	[Myotis lucifugus]	NCBI Reference Sequence:	XP_023609439.1	89.0%
Bat#12	[Phyllostomus discolor]	NCBI Reference Sequence:	XP_028378317.1	87.0%
Bat#13	[Hipposideros armiger]	NCBI Reference Sequence:	XP_019522936.1	89.0%
Bat#14	[Hipposideros armiger]	NCBI Reference Sequence:	XP_019522943.1	89.0%
Bat#15	[Hipposideros armiger]	NCBI Reference Sequence:	XP_019522954.1	89.0%
Pangolin#1	[Manis javanica]	NCBI Reference Sequence:	XP_017505746.1	91.0%
Pangolin#2	[Manis javanica]	NCBI Reference Sequence:	XP_017505752.1	91.0%
Snake#1	[Notechis scutatus]	NCBI Reference Sequence:	XP_026530754.1	75.0%
Snake#2	[Thamnophis elegans]	NCBI Reference Sequence:	XP_032082934.1	74.0%

https://blast.ncbi.nlm.nih.gov/Blast.cgi

Figure 2 Amino acid homology of Angiotensin Converting Enzyme 2 (ACE2) between human and animals

Similarity of amino acid Sequence identities for human Angiotensin Converting Enzyme 2 (ACE2) (protein accession number NP_001358344.1) compared with ACE2of other animal species including dogs, cats, bats, pangolins and snakes. Detailed information including protein accession numbers of ACE2 of other species can be found in the supplementary material.

Hayashi *et al.* Figure 3

angiotensin-converting enzyme 2 precursor [Homo sapiens]

NCBI Reference Sequence: **NP_001358344.1**

353..357 kgdfr /region_name="Interaction with SARS-CoV spike glycoprotein"
/note="propagated from UniProtKB/Swiss-Prot (Q9BYF1.2)"

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Dog#1    301 wdarkifkea ekffvsvglp nmtqefwgns mltepsdsrk vvchptawdl gkgdfrikmc
Dog#2    301 wdarkifkea ekffvsvglp nmtqefwens mltepsdsrk vvchptawdl gkgdfrikmc
Dog#3    301 wdarkifkea ekffvsvglp nmtqefwens mltepsdsrk vvchptawdl gkgdfrikmc
Dog#4    181 darkifkeae kffvsvglpn mtqefwensm ltepsdsrkv vchptawdlg kgdfrikmct
Cat#1    301 nqswdarrif keaekffvsv glpnmtqgfw ensmltepgd srkvvchpta wdlgkgdfri
Cat#2    301 swdarrifke aekffvsvgl pnmtqgfwen smltepgdsr kvvchptawd lgkgdfrikm
Tiger#1  291 nqswdarrif keaekffvsv glpnmtqgfw ensmltepgn sqkvvchpta wdlgkgdfri
Bat#1    301 wdaekifkea ekfyisvglp smtpgfwnns mltepgdgrk vvchptawdl gkgdfrikmc
Bat#2    301 wdaekifkea ekfyisvglp smtpgfwnns mltepgdgrk vvchptawdl gkgdfrikmc
Bat#3    301 wdaekifkea ekfyisvglp smtpgfwnns mltepgdgrk vvchptawdl gkgdfrikmc
Bat#4    301 wdaekifkea ekfyisvglp smtpgfwnns mltepgdgrk vvchptawdl gkgdfrikmc
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Bat#6    231 dqswdaqrif keaekffksv glfsmtqgfw dnsmltkpdd grevvchpta wdlgnkdfri
Bat#7    301 wdaekifkea ekfymsvglp smtpgfwnns mltepgdgrk vvchptawdl gkndfrikmc
Bat#8    301 wdaekifkea ekfymsvglp smtpgfwnns mltepgdgrk vvchptawdl gkndfrikmc
Bat#9    301 wdaekifkea ekfyisvglp smtpgfwnns mltepgdgrk vvchptawdl gkgdfrikmc
Bat#10   301 wdaekifkea ekfyisvglp smtpgfwnns mltepgdgrk vvchptawdl gkgdfrikmc
Bat#11   301 wdaekifkea ekfyisvglp smtpgfwnns mltepgdgrk vvchptawdl gkgdfrikmc
Bat#12   301 aqrifkeaek ffvsvglfnm tqgfwdnsml tkpddgrevv chptawdlgk kdfrikmctk
Bat#13   301 kwdakkifqe aekffvsvgl pnmtkgfwen smltepgdgr kvvchptawd lgkgdfrikm
Bat#14   301 kwdakkifqe aekffvsvgl pnmtkgfwen smltepgdgr kvvchptawd lgkgdfrikm
Bat#15   301 kwdakkifqe aekffvsvgl pnmtkgfwen smltepgdgr kvvchptawd lgkgdfrikm
Pan#1    301 twdanrifke aekffvsvgl pkmtqtfwen smltepgdgr kvvchptawd lgkhdfrikm
Pan#2    301 twdanrifke aekffvsvgl pkmtqtfwen smltepgdgr kvvchptawd lgkhdfrikm
Sna#1    361 ekkwtvdsif kaaehffisi glfnmtesfw knsmleepkd grkvvchpta wdmgkedyri
Sna#2    321 tkkwtvnsif kaaeqfftsi glfpmtdnfw nnsmleepkd grkvvchpta wdmgkkdyri
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Figure 3 Amino acid sequence alignment of the binding region of angiotensin converting enzyme 2 (ACE2) as receptor for SARS-CoV-2 spike glycoprotein and its phylogeny

The binding region of Angiotensin Converting Enzyme 2 (ACE2) and the homologous region of ACE2 of other animal speciesincluding dogs, cats, bats, pangolins and snakes are indicated by red words. The key 5amino acid residues KGDFR involved in theinteraction with human SARS-CoV-2 spike glycoprotein are marked with the red bold words. Detailed information including protein accession numbers of ACE2 of other animal species can be found in the supplementary material.