Received: 13 April 2020 | Revised: 1 August 2020 | Accepted: 2 August 2020

DOI: 10.1111/tbed.13773

RAPID COMMUNICATION

Transboundary and Emerging Diseases WILEY

ACE2 isoform diversity predicts the host susceptibility of SARS-CoV-2

Shan Gao[1] | Junwen Luan[1] | Haoran Cui[1] | Leiliang Zhang[1,2]

[1]Institute of Basic Medicine, The First Affiliated Hospital of Shandong First Medical University, Jinan, China

[2]Science and Technology Innovation Center, Shandong First Medical University & Shandong Academy of Medical Sciences, Jinan, China

Correspondence
Leiliang Zhang, Institute of Basic Medicine, The First Affiliated Hospital of Shandong First Medical University, Jinan 250062, Shandong, China.
Email: armzhang@hotmail.com

Funding information
National Key Plan for Research and Development of China:2016YFD0500300; National Natural Science Foundation of China, Grant/Award Number: 81871663 and 81672035; the Innovation Project of Shandong Academy of Medical Sciences; Academic promotion programme of Shandong First Medical University, Grant/Award Number: 2019LJ001

Abstract

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) causes the ongoing coronavirus disease 2019 (COVID-19) pandemic. Angiotensin-converting enzyme 2 (ACE2) is the functional receptor for SARS-CoV-2. In our current study, we found that two types of deficient ACE2 isoforms from different mammals compete with full-length ACE2 for association with S protein. One type of ACE2 is a natural soluble isoform, the other type of ACE2 only associates with one loop of the receptor-binding domain (RBD) of the SARS-CoV-2 S protein. Mammals with either type of ACE2 will be deficient in support of SARS-CoV-2 entry. By combining S recognition and isoform analysis of ACE2, we predict that felids, mustelids, hamsters, and sheep are susceptible to SARS-CoV-2, while canids, swines, cattle, and goats are not permissive for SARS-CoV-2. Thus, the differential susceptibilities of mammals with SARS-CoV-2 infection could be partially explained by the ACE2 isoform diversity. Our findings will shed important light on predicting the host range of other zoonotic viruses.

KEYWORDS

ACE2, COVID-19, host susceptibility, isoform, SARS-CoV-2

1 | INTRODUCTION

Corona virus disease 2019 (COVID-19) caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is an ongoing global pandemic, with over 1.6 million confirmed cases as of April 10, 2020. Bats (*Rhinolophus sinicus*) are well accepted as SARS-CoV-2 original hosts (Zhou et al., 2020). However, the host ranges and intermediate hosts for SARS-CoV-2 are not clear.

Spike (S) protein determines the host range of SARS-CoV-2 because of its role in recognizing human angiotensin-converting enzyme 2 (ACE2) as the virus receptor (Zhou et al., 2020). Functional ACE2 is assembled as heterodimer, with the C-terminal collectrin-like domain of ACE2 mediating dimerization (Yan et al., 2020). The SARS-CoV-2 receptor-binding domain (RBD) is recognized by the extracellular N-terminal peptidase domain of ACE2. Several studies predicted the host range mainly based on the potential association of S with ACE2 from different species (Li, Qiao, & Zhang, 2020; Luan, Jin, Lu, & Zhang, 2020; Luan, Lu, Jin, & Zhang, 2020). However, the recognition of ACE2 does not guarantee that this ACE2 would support SARS-CoV-2 infection. Susceptibility of potential hosts may not be only limited by the binding ability of ACE2 to S protein. For instance, although ACE2 from both feline and canine species is predicted to associate with S, SARS-CoV-2 was prone to infect felids rather than canine species (Shi et al., 2020). This prominent phenomenon indicates that other factors are involved in determining the host range of this virus.

Until now, the known mammalian hosts which support SARS-CoV-2 infection are humans, felines, ferrets, hamsters, and rhesus macaques, while ACE2 from canines and swines are non-permissive for SARS-CoV-2 infection (Chan et al., 2020; Deng et al., 2020; Shi et al., 2020). Alternative splicing increases the complexity of mammalian proteins,

Shan Gao and Junwen Luan are co-first authors.

leading to existence of multiple isoforms of a single protein. One type of alternative splicing causes insertions or deletions (indels). The other type contains exon substitutions. Alternative splicing with indels generates isoforms of different sizes. In this study, we found that ACE2 proteins from different species have isoforms deficient in support of SARS-CoV-2 entry. The differential SARS-CoV-2 susceptibilities of mammals could be partially explained by the ACE2 isoform diversity.

2 | MATERIALS AND METHODS

2.1 | Sequence analysis of ACE2 isoforms

The isoforms of ACE2 from selected mammals were searched in UniProt (https://www.uniprot.org/). These ACE2 proteins with their corresponding species are listed as follows: five canine (*Canis lupus*) isoforms (UniID: dog1, J9P7Y2; dog2, F1P7C5; dog3, C7ECV0; dog4, A0A5F4BS93; dog5, A0A5F4CXG9), three swine (*Sus scrofa*) isoforms (UniID: pig1, K7GLM4; pig2, A0A4X1UWQ8; pig3, A0A4X1UXU4), three goat (*Capra hircus*) isoforms (UniID: goat1, A0A452EVJ5; goat2, A0A452EVU0; goat3, A0A452EVM2), two cattle (*Bos taurus*) isoforms (UniID: cow1, Q58DD0; cow2, A0A452DJE0), two hamster (*Mesocricetus auratus*) isoforms (UniID: hamster1, A0A1U7QTA1; hamster2, C7ECV1), one ferret (*Mustela putorius furo*) isoform (UniID: Q2WG88), one rhesus macaque (*Macaca mulatta*) isoform (UniID: F7AH40), one sheep (*Ovis aries*) isoform (UniID: W5PSB6), three feline (*Feliscatus*) isoforms (UniID: cat1, Q56H28; cat2, A0A5F5XDN9; cat3, A0A384DV19), one human (*Homo sapiens*) isoform (UniID: Q9BYF1), two lagomorph (*Oryctolagus cuniculus*) isoforms (UniID: rabbit1, G1TEF4; rabbit2, C7ECU4), nine polar bear (*Ursus maritimus*) isoforms (UniID: polar bear1, A0A452TT30; polar bear2, A0A384CIJ9; polar bear3, A0A452TT37; polar bear4, A0A452TTF7; polar bear5, A0A452TT98; polar bear6, A0A452TTE2; polar bear7, A0A452TT60; polar bear8, A0A452TTD2; polar bear9, A0A452TTE1), one crab-eating macaque (*Macaca fascicularis*) isoform (UniID: A0A2K5X283), one golden snub-nosed monkey (*Rhinopithecus roxellana*) isoform (UniID: A0A2K6NFG7), one drill (*Mandrillus leucophaeus*) isoform (UniID: A0A2K5ZV99), one sperm whale (*Physeter macrocephalus*) isoform (UniID: A0A2Y9S5T9), one beluga whale (*Delphinapterus leucas*) isoform (UniID: A0A2Y9M9H3), two Sumatran orangutan (*Pongo abelii*) isoforms (UniID: orangutan1, Q5RFN1; orangutan2, H2PUZ5), two Ord's kangaroo rat (*Dipodomys ordii*) isoforms (UniID: kangaroo rat1, A0A1S3GHT7; kangaroo rat2, A0A1S3GFD6), two guinea pig (*Cavia porcellus*) isoforms (UniID: guinea pig1, H0VSF6; guinea pig2, C7ECU4), two chimpanzee (*Pan troglodytes*) isoforms (UniID: chimpanzee1, A0A2J8KU96; chimpanzee2, A0A2I3S8E3), and two pygmy chimpanzee (*Pan paniscus*) isoforms (UniID: pygmy chimpanzee1, A0A2R9BKD8; pygmy chimpanzee2, A0A2R9BJK0). Analysis of ACE2 proteins was conducted by using Molecular Evolutionary Genetics Analysis (MEGA) version X (Kumar, Stecher, Li, Knyaz, & Tamura, 2018).

2.2 | Structure simulation of ACE2-S complex

Based on the structure of full-length human ACE2 with SARS-CoV-2 S RBD (Protein Data Bank [PDB]: 6M17) (Yan et al., 2020), the structure of SARS-CoV-2 S RBD and ACE2 isoforms from selected mammals were simulated by the SWISS-MODEL online server (Waterhouse et al., 2018) and analysed by Chimera software ver. 1.14 (Pettersen et al., 2004).

3 | RESULTS AND DISCUSSION

ACE2 isoforms from canine species were investigated. There are five canine isoforms (Table 1), one of which (dog4) lacks a transmembrane domain, and so it is a soluble canine ACE2 (Figure 1a). Soluble human ACE2 was recently reported to block the interaction between ACE2 and SARS-CoV-2 S (Monteil et al., 2020). The simulated structure shows that dog4 could associate with SARS-CoV-2 S (Figure 1b). Based on this, we think that dog4 could play a competitive inhibitory role for interaction between full-length ACE2 and SARS-CoV-2 S. Soluble ACE2 was no longer to mediate subsequent viral entry. In this sense, the binding possibility of RBD to "effective" canine ACE2 is reduced, leaving canines not susceptible for SARS-CoV-2 infection, which is consistent with the experimental evidence (Shi et al., 2020).

Swines have one ACE2 isoform (pig3) lacking 1–122 residues in the N terminus of ACE2, which is critical for binding one of two loops of SARS-CoV-2 RBD protruding into the interface (Figure 1c,d) (Lan et al., 2020). This pig3 isoform only associates with the other loop of SARS-CoV-2 RBD. Thus, it would inhibit the interaction between full-length ACE2 and S. In a recent study, SARS-CoV-2 was not detected in samples collected from virus-inoculated pigs (Shi et al., 2020). This result suggested that swines are not susceptible for SARS-CoV-2, which is supportive of our prediction based on isoform analysis.

Humans, rhesus macaques, crab-eating macaques, and ferrets have only one ACE2 isoform (Table 1). Their ACE2 proteins maintain the majority of critical amino acids (AAs) for associating with S. For other species which have more than one ACE2 isoform, they can be divided into two categories. The first category has AA deletions or substitutions outside the binding domain. Hamsters have two ACE2 isoforms with the short one containing a deletion in the cytoplasmic tail. Previous work showed that the cytoplasmic tail is not required for the receptor function of SARS-CoV (Inoue et al., 2007). We propose that the cytoplasmic tail of ACE2 is not critical for SARS-CoV-2 infection, thus this deletion will not have much impact on receptor binding. Another species of this category are felids which have three isoforms. However, these three isoforms have similar lengths and show high identity in sequences. They maintain the critical residues for S binding (Table 1), and only isoform 3 has four AA substitutions. We speculate that all of these isoforms have similar binding affinities for SARS-CoV-2 S. The above animals are predicted to support SARS-CoV-2

TABLE 1 SARS-CoV-2 susceptibilities of selected mammals based on ACE2 isoforms

Species	Host range	Isoforms	UniID	Length (AA)	Extracellular domain	S binding	Transmembrane domain
Canine (*Canis lupus*)	−	1	J9P7Y2	804	Intact	+	739–761
		2	F1P7C5	765	667–705 deleted	+	700–722
		3	C7ECV0	804	Intact	+	739–761
		4	A0A5F4BS93	739	706–738 deleted	+	None
		5	A0A5F4CXG9	777	Intact	+	739–761
Pig (*Sus scrofa*)	−	1	K7GLM4	805	Intact	+	740–762
		2	A0A4X1UWQ8	805	Intact	+	740–762
		3	A0A4X1UXU4	683	1–122 deleted	−	618–640
Goat (*Capra hircus*)	−	1	A0A452EVJ5	804	Intact	+	739–761
		2	A0A452EVU0	787	122–139 deleted	+	722–744
		3	A0A452EVM2	743	1–61 deleted	−	678–700
Cattle (*Bos taurus*)	−	1	Q58DD0	804	Intact	+	739–761
		2	A0A452DJE0	743	1–61 deleted	−	678–700
Hamster (*Mesocricetus auratus*)	+	1	A0A1U7QTA1	805	Intact	+	740–762
		2	C7ECV1	785	786–805 deleted	+	740–762
Ferret (*Mustela putorius furo*)	+	1	Q2WG88	805	Intact	+	740–762
Rhesus macaque (*Macaca mulatta*)	+	1	F7AH40	805	Intact	+	740–762
Crab-eating macaque (*Macaca fascicularis*)	+	1	A0A2K5X283	805	Intact	+	740–762
Golden snub-nosed monkey (*Rhinopithecus roxellana*)	+	1	A0A2K6NFG7	805	Intact	+	740–762
Drill (*Mandrillus leucophaeus*)	+	1	A0A2K5ZV99	805	Intact	+	740–762
Sperm whale (*Physeter macrocephalus*)	+	1	A0A2Y9S5T9	804	Intact	+	740–762
Beluga whale (*Delphinapterus leucas*)	+	1	A0A2Y9M9H3	804	Intact	+	739–761
Sumatran orangutan (*Pongo abelii*)	+	1	Q5RFN1	805	Intact	+	741–763
		2	H2PUZ5	802	Intact	+	738–760
Ord's kangaroo rat (*Dipodomys ordii*)	−	1	A0A1S3GHT7	805	Intact	+	741–763
		2	A0A1S3GFD6	740	706–770 deleted	+	None
Guinea pig (*Cavia porcellus*)	+	1	H0VSF6	794	Intact	+	729–751
		2	C7ECU4	789	Intact	+	740–762
Chimpanzee (Pan troglodytes)	+	1	A0A2J8KU96	805	Intact	+	741–763
		2	A0A2I3S8E3	694	556–666 deleted	+	630–652
Pygmy chimpanzee (*Pan paniscus*)	+	1	A0A2R9BKD8	805	Intact	+	741–763
		2	A0A2R9BJK0	694	556–666 deleted	+	630–652
Sheep (*Ovis aries*)	+	1	W5PSB6	805	Intact	+	740–762
Feline (*Feliscatus*)	+	1	Q56H28	805	Intact	+	740–762
		2	A0A5F5XDN9	805	Intact	+	740–762
		3	A0A384DV19	803	Intact	+	738–760
Human (*Homo sapiens*)	+	1	Q9BYF1	805	Intact	+	741–761
Lagomorph (*Oryctolagus cuniculus*)	+	1	G1TEF4	805	Intact	+	741–763
		2	C7ECU4	783	784–805 deleted	+	741–763

(Continues)

TABLE 1 (Continued)

Species	Host range	Isoforms	UniID	Length (AA)	Extracellular domain	S binding	Transmembrane domain
Polar bear (*Ursus maritimus*)	–	1	A0A452TT30	805	Intact	+	740–762
		2	A0A384CIJ9	790	1–15 deleted	+	725–747
		3	A0A452TT37	784	1–21 deleted	+	719–741
		4	A0A452TTF7	774	1–22 28–36 deleted	–	709–731
		5	A0A452TT98	798	1–7 deleted	+	733–755
		6	A0A452TTE2	780	1–16 28–36 deleted	–	715–737
		7	A0A452TT60	790	1–15 deleted	+	725–747
		8	A0A452TTD2	796	1–9 deleted	+	731–753
		9	A0A452TTE1	776	1–20 28–36 deleted	–	711–733

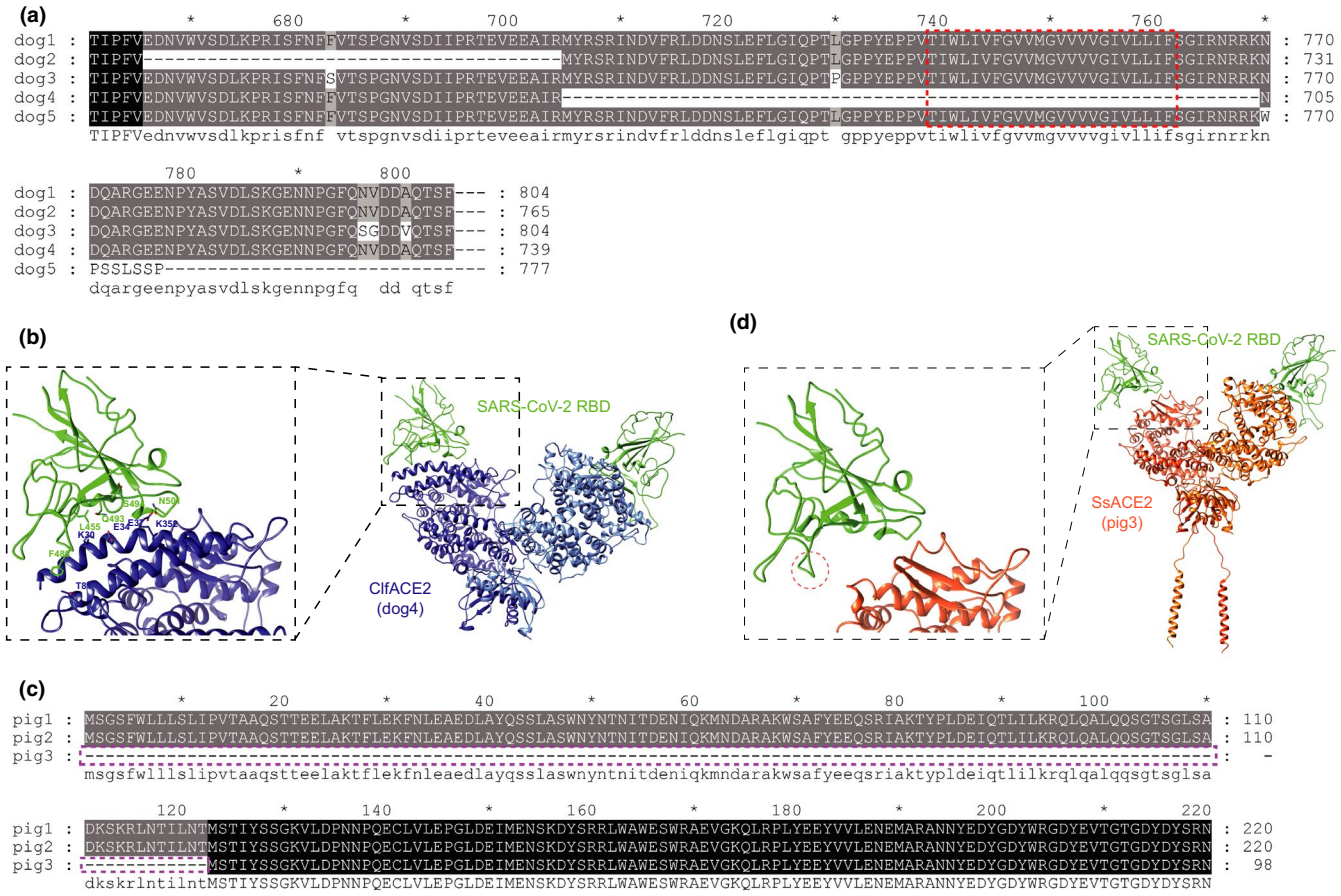

FIGURE 1 Alignment of canine and swine ACE2 isoforms showing that some isoforms lack the critical region for receptor function. (a) Five canine isoforms (UniID: dog1, J9P7Y2; dog2, F1P7C5; dog3, C7ECV0; dog4, A0A5F4BS93; dog5, A0A5F4CXG9). The dotted box highlights the transmembrane region which is predicted by TMHMM 2.0. (b) Simulated structure of ClfACE2 (dog4) and SARS-CoV-2 S RBD. Two SARS-CoV-2 S RBDs are coloured in green and lime green, ClfACE2 dimer in medium blue and cornflower blue. The inset shows the detailed analysis of interface between RBD and ACE2. (c) Three swine isoforms (UniID: pig1, K7GLM4; pig2, A0A4X1UWQ8; pig3, A0A4X1UXU4). Dotted box shows missing region in the extracellular domain. (d) Simulated structure of SsACE2 (pig3) and SARS-CoV-2 S RBD. The colour of SARS-CoV2 S RBD is the same as in Figure 1b. SsACE2 dimer is coloured in orange and orange red. The inset shows the detailed analysis of interface between RBD and ACE2. The dotted cycle shows the loop that does not associate with SsACE2 (pig3). ACE2, angiotensin-converting enzyme 2; RBD, receptor-binding domain; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2 [Colour figure can be viewed at wileyonlinelibrary.com]

infection, which is consistent with the experimental evidence (Chan et al., 2020; Deng et al., 2020; Shi et al., 2020).

Next, we predicted the SARS-CoV-2 susceptibilities of other mammals based on ACE2 isoforms. The only isoform in sheep, golden snub-nosed monkeys, drills, sperm whales, and beluga whales matches the majority of key residues in human ACE2 for SARS-CoV-2 binding (Table 1), which is predicted to support SARS-CoV-2 infection (Li et al., 2020; Luan, Jin, et al., 2020). Sumatran orangutans and guinea pigs have two isoforms with minor AA differences, which were both supportive for virus entry (Table 1). Lagomorphs have two isoforms, with the short one containing a deletion in the cytoplasmic tail (Table 1). Similar to the case of hamsters, lagomorphs are predicted to support SARS-CoV-2 S infection. Both chimpanzees and pygmy chimpanzees have two isoforms, with one shorter than the canonical one. Since deletion at the extracellular domain in the short isoform does not contain critical AAs for S binding, we predict that the two isoforms can equally mediate SARS-CoV2 infection (Table 1). As for Ord's kangaroo rats, the deletion of ACE2 is the same as that of canids, which leads to a lack of the transmembrane domain. We hypothesize that this soluble

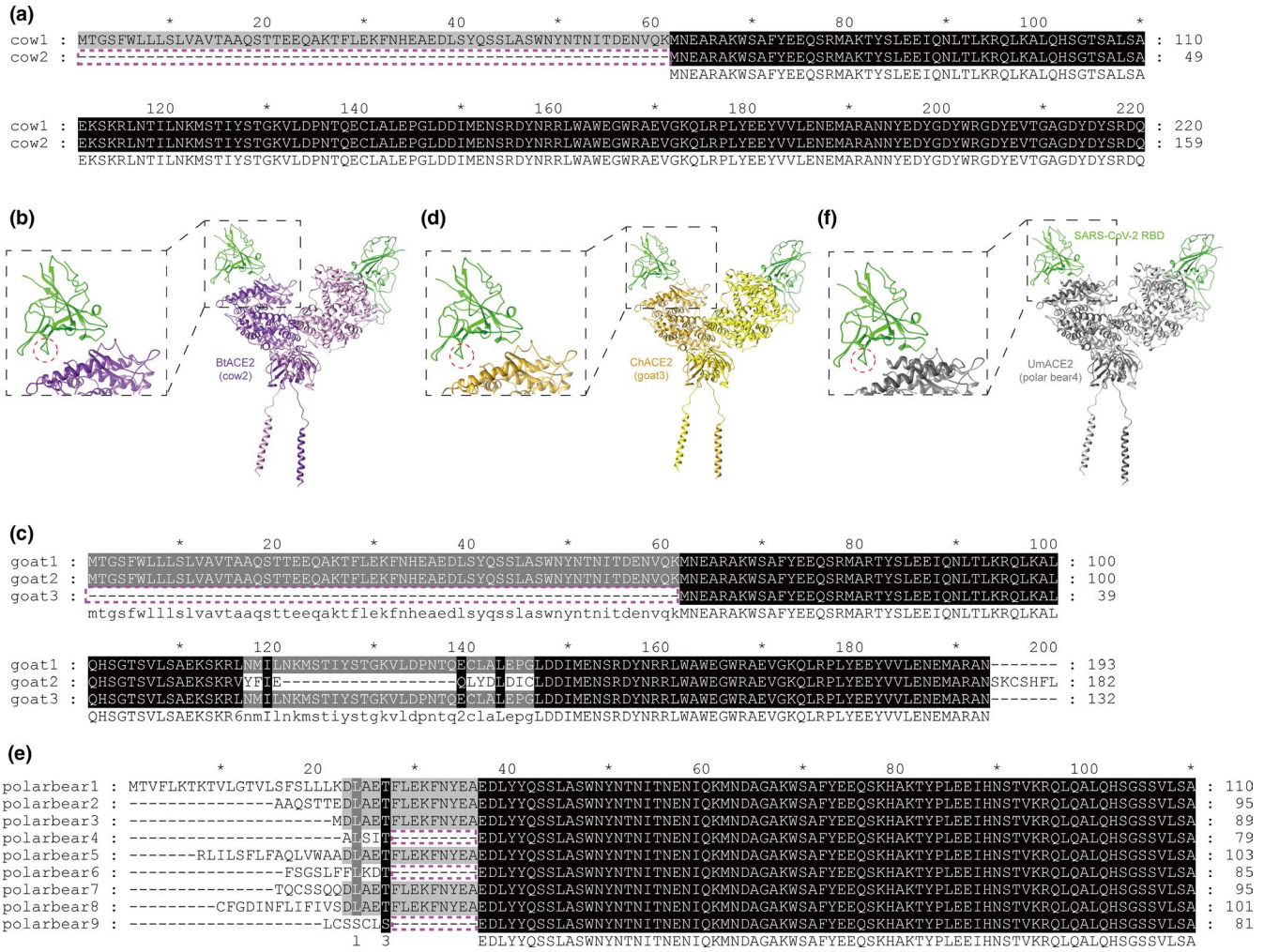

FIGURE 2 Alignment of ACE2 isoforms from cattle, goats, and polar bears showing that some isoforms lack the critical region for receptor function. (a) Two cattle isoforms (UniID: cow1, Q58DD0; cow2, A0A452DJE0). Dotted box shows missing region in the extracellular domain comprising critical residues for S binding. (b) Simulated structure of BtACE2 (cow2) and SARS-CoV-2 S RBD. The colour of SARS-CoV2 S RBD is the same as in Figure 1b. BtACE2 dimer is coloured in purple and plum. The dotted cycle is the loop that does not associate with BtACE2 (cow2). (c) Three goat isoforms (UniID: goat1, A0A452EVJ5; goat2, A0A452EVU0; goat3, A0A452EVM2). Dotted box shows missing region in the extracellular domain comprising critical residues for S binding. (d) Simulated structure of ChACE2 (goat3) and SARS-CoV-2 S RBD. The colour of SARS-CoV2 S RBD is the same as in Figure 1b. ChACE2 dimer is coloured in gold and yellow. The dotted cycle is the loop that does not associate with ChACE2 (goat3). (e) Nine polar bear isoforms (UniID: polarbear1, A0A452TT30; polarbear2, A0A384CIJ9; polarbear3, A0A452TT37; A0A452EVM2; polarbear4, A0A452TTF7; polarbear5, A0A452TT98; polarbear6, A0A452TTE2; polarbear7, A0A452TT60; polarbear8, A0A452TTD2; polarbear9, A0A452TTE1). Dotted box shows missing region in the extracellular domain comprising critical residues for S binding. (f) Simulated structure of UmACE2 (polar bear4) and SARS-CoV-2 S RBD. The colour of SARS-CoV2 S RBD is the same as in Figure 1b. UmACE2 dimer is coloured in grey and dim grey. The dotted cycle is the loop that does not associate with UmACE2 (polar bear4). ACE2, angiotensin-converting enzyme 2; RBD, receptor-binding domain; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2 [Colour figure can be viewed at wileyonlinelibrary.com]

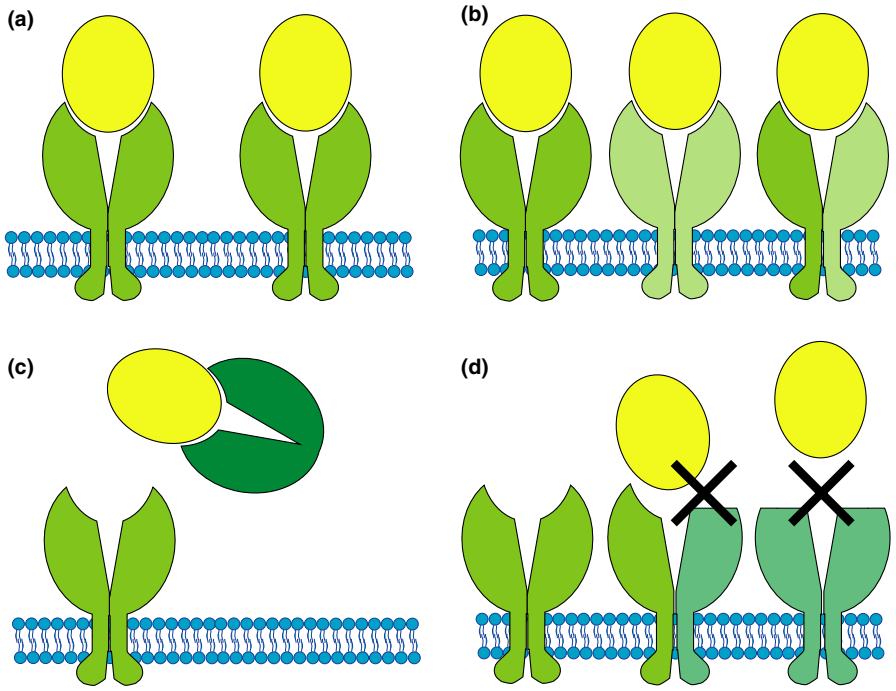

FIGURE 3 Schematics of four representative species containing different types of ACE2 isoforms. (a) Humans have a single type of ACE2. (b). Species such as felines have more than one isoform, all of which are able to bind S. (c) Canines have four ACE2 isoforms. One of them loses the transmembrane domain; so it exists in soluble form and competitively binds S with its intact ectodomain. (d) Species such as pigs have multiple isoforms. One or more of them lack N-terminal fragment containing critical AAs for RBD binding and only recognize one loop of RBD. Therefore, these isoforms of ACE2 will compete with full-length ACE2 for association of S. The yellow oval represents S trimer, and ACE2 dimers from different species are coloured in green or cyan. AA, amino acid; ACE2, angiotensin-converting enzyme 2; RBD, receptor-binding domain [Colour figure can be viewed at wileyonlinelibrary.com]

isoform may also protect kangaroo rats from SARS-CoV-2 infection (Table 1).

Cattle have two ACE2 isoforms and goats have three (Table 1). Both of them have one isoform (cow2, goat3) that lacks 61 residues in the N terminus, which is critical for interacting with one of two loops of S protein (Figure 2a–d).

Polar bears have nine ACE2 isoforms (Table 1). Isoform 4, isoform 6, and isoform 9 all contain a deletion in 28–36 residues, which is critical to associate with one of two loops of SARS-CoV-2 S (Figure 2e,f). Those isoforms will compete with full-length ACE2 for association with S protein, leading to reduced SARS-CoV-2 entry in cattle, goats, and polar bears.

Our previous studies predicted the host range based on the interaction between SARS-CoV-2 S and full-length ACE2 (Luan, Jin, et al., 2020; Luan, Lu, et al., 2020). However, full-length ACE2 with S-binding ability alone does not guarantee the host susceptibility because some species encode multiple ACE2 isoforms. In this current study, we classified four types of mammals based on ACE2 isoforms analysis (Figure 3). The first ones have a single ACE2, which maintains the intact receptor function (Figure 3a). The second ones, such as felids, have more than one isoform, all of which are capable to bind S (Figure 3b). The third ones, such as canids, have one ACE2 isoform that loses the transmembrane domain. This soluble form of ACE2 maintains the capacity of S binding, while it fails to mediate virus entry. It will compete with full-length ACE2 for association of S (Figure 3c). Swines, the representatives of the fourth type of mammals, contain one or more isoforms lacking N-terminal fragments containing critical AAs for RBD binding. Therefore, the deficient ACE2 isoforms will compete with full-length ACE2 for association of S and inhibit its normal function (Figure 3d). The first and second types of mammals will likely be permissive for SARS-CoV-2 infection, while the third and fourth types of mammals will be non-permissive or less permissive for SARS-CoV-2 infection.

In conclusion, we propose that ACE2 isoform diversity could play an important role in determining the host range of SARS-CoV-2. We predict that felines, ferrets, hamsters, rhesus macaques, sheep, lagomorphs, crab-eating macaques, golden snub-nosed monkeys, drills, sperm whales, beluga whales, guinea pigs, chimpanzees, and pygmy chimpanzees are susceptible to SARS-CoV-2, while canines, swines, cattle, goats, polar bears, and Ord's kangaroo rats are resistant to SARS-CoV-2. Our findings not only explain the known potential hosts for SARS-CoV-2 but also predict the SARS-CoV-2 susceptibilities of other mammals. The strategy of isoform analysis will provide novel important clues to predict the host range of other zoonotic viruses.

ACKNOWLEDGEMENTS

This work was supported by grants from the National Key Plan for Research and Development of China (2016YFD0500300),

the National Natural Science Foundation of China (81871663 and 81672035), the Innovation Project of Shandong Academy of Medical Sciences, and the Academic Promotion Programme of Shandong First Medical University (2019LJ001).

CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest.

ETHICAL APPROVAL

The authors confirm that the ethical policies of the journal, as noted on the journal's author guidelines page, have been adhered to. No ethical approval was required as this report solely contains bioinformatics studies.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ORCID

Leiliang Zhang https://orcid.org/0000-0002-7015-9661

REFERENCES

Chan, J.-W., Zhang, A. J., Yuan, S., Poon, V.-M., Chan, C.-S., Lee, A.-Y., … Yuen, K.-Y. (2020). Simulation of the clinical and pathological manifestations of Coronavirus Disease 2019 (COVID-19) in golden Syrian hamster model: Implications for disease pathogenesis and transmissibility. *Clinical Infectious Diseases*. https://doi.org/10.1093/cid/ciaa325

Deng, W., Bao, L., Gao, H., Xiang, Z., Qu, Y., Song, Z., & Qin, C. (2020). Ocular conjunctival inoculation of SARS-CoV-2 can cause mild COVID-19 in Rhesus macaques. *Nat Commun*, *11*(1), 4400–. https://doi.org/10.1101/2020.03.13.990036

Inoue, Y., Tanaka, N., Tanaka, Y., Inoue, S., Morita, K., Zhuang, M., … Sugamura, K. (2007). Clathrin-dependent entry of severe acute respiratory syndrome coronavirus into target cells expressing ACE2 with the cytoplasmic tail deleted. *Journal of Virology*, *81*(16), 8722–8729. https://doi.org/10.1128/jvi.00253-07

Kumar, S., Stecher, G., Li, M., Knyaz, C., & Tamura, K. (2018). MEGA X: Molecular evolutionary genetics analysis across computing platforms. *Molecular Biology and Evolution*, *35*(6), 1547–1549. https://doi.org/10.1093/molbev/msy0964990887

Lan, J., Ge, J., Yu, J., Shan, S., Zhou, H., Fan, S., … Wang, X. (2020). Structure of the SARS-CoV-2 spike receptor-binding domain bound to the ACE2 receptor. *Nature*, *581*, 215–220. https://doi.org/10.1038/s41586-020-2180-5

Li, R., Qiao, S., & Zhang, G. (2020). Analysis of angiotensin-converting enzyme 2 (ACE2) from different species sheds some light on cross-species receptor usage of a novel coronavirus 2019-nCoV. *Journal of Infection*, *80*(4), 469–496. https://doi.org/10.1016/j.jinf.2020.02.013

Luan, J., Jin, X., Lu, Y., & Zhang, L. (2020). SARS-CoV-2 spike protein favors ACE2 from Bovidae and Cricetidae. *Journal of Medical Virology*. https://doi.org/10.1002/jmv.25817

Luan, J., Lu, Y., Jin, X., & Zhang, L. (2020). Spike protein recognition of mammalian ACE2 predicts the host range and an optimized ACE2 for SARS-CoV-2 infection. *Biochemical and Biophysical Research Communications*, *526*(1), 165–169. https://doi.org/10.1016/j.bbrc.2020.03.047

Monteil, V., Kwon, H., Prado, P., Hagelkrüys, A., Wimmer, R. A., Stahl, M., … Penninger, J. M. (2020). Inhibition of SARS-CoV-2 infections in engineered human tissues using clinical-grade soluble human ACE2. *Cell*, *181*, 905–913.e7, https://doi.org/10.1016/j.cell.2020.04.004

Pettersen, E. F., Goddard, T. D., Huang, C. C., Couch, G. S., Greenblatt, D. M., Meng, E. C., & Ferrin, T. E. (2004). UCSF Chimera–a visualization system for exploratory research and analysis. *Journal of Computational Chemistry*, *25*(13), 1605–1612. https://doi.org/10.1002/jcc.20084

Shi, J., Wen, Z., Zhong, G., Yang, H., Wang, C., Huang, B., … Bu, Z. (2020). Susceptibility of ferrets, cats, dogs, and other domesticated animals to SARS–coronavirus 2. *Science*, *368*(6494), 1016–1020. https://doi.org/10.1126/science.abb7015

Waterhouse, A., Bertoni, M., Bienert, S., Studer, G., Tauriello, G., Gumienny, R., … Schwede, T. (2018). SWISS-MODEL: Homology modelling of protein structures and complexes. *Nucleic Acids Research*, *46*(W1), W296–W303. https://doi.org/10.1093/nar/gky427

Yan, R., Zhang, Y., Li, Y., Xia, L., Guo, Y., & Zhou, Q. (2020). Structural basis for the recognition of SARS-CoV-2 by full-length human ACE2. *Science*, *367*(6485), 1444–1448. https://doi.org/10.1126/science.abb2762

Zhou, P., Yang, X.-L., Wang, X.-G., Hu, B., Zhang, L., Zhang, W., … Shi, Z.-L. (2020). A pneumonia outbreak associated with a new coronavirus of probable bat origin. *Nature*, *579*(7798), 270–273. https://doi.org/10.1038/s41586-020-2012-7

How to cite this article: Gao S, Luan J, Cui H, Zhang L. ACE2 isoform diversity predicts the host susceptibility of SARS-CoV-2. *Transbound Emerg Dis*. 2021;68:1026–1032. https://doi.org/10.1111/tbed.13773