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RESEARCH ARTICLE

ACE2 Expression Patterns Across Mammals and Key Findings for SARS-CoV-2 Model Development for Human and Animal Research

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ABSTRACT

The novel coronavirus (SARS-CoV-2), responsible for over 40 million infections and one million deaths globally, has posed a significant public health challenge. As this virus infects both humans and animals, understanding the interactions between SARS-CoV-2 and its potential animal hosts is crucial for both public and veterinary health. With the discovery of angiotensin-converting enzyme 2 (ACE2) as the virus functional receptor, efforts to develop antiviral treatments and vaccines have advanced. However, the resistance of mice to SARS-CoV-2 has intensified the search for alternative animal models. In this study phylogenetic analysis was conducted by utilizing MEGA X (v10.1.7) with the JTT substitution model and 100 bootstrap replicates. Protein sequence alignment was performed via ClustalW2. The distribution of ACE2 expression was analyzed utilizing the GTEx portal, GEO database, and the ggplot2 software for statistical analysis. ACE2 expression across several organs was assessed using the Human Protein Atlas, whereas protein-protein interactions were investigated through the STRING database. This study analyzes ACE2 evolutionary history and expression across mammalian species, identifying the crab-eating macaque as a strong candidate due to its five identical ACE2 hotspot residues with humans. Other species, including cattle, pigs, ferrets, and cats, share varying degrees of similarity. ACE2 expression patterns, particularly in lung and colon tissues, are also highlighted across species. These findings offer valuable insights for selecting optimal animal models to accelerate COVID-19 research and therapeutic development.

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INTRODUCTION

The epidemic of COVID-19 was acknowledged as a "public health emergency of international concern (PHEIC)" by the World Health Organization (WHO) on January 30th, 2020 (Pereira *et al.*, 2024). The phylogenetic study of entire genomic sequence of SARS-CoV-2 presented that these two sets of SARS-CoV-2 genotypes formed coherent clusters within the genus Beta-coronavirus. Infecting wild animals, livestock, and people, beta-coronavirus is an RNA-enveloped virus that causes sporadic epidemics including both SARS and MERS, which are very dangerous respiratory illnesses (Kaur *et al.*, 2021). A current investigation revealed that SARS-CoV-2 has a genetic similarity of 96% at the complete molecular basis with RaTG13, a virus found in bats (Gulati *et al.*, 2023). While this data suggests that bats may have served as the initial reservoir, the precise mode of transmission and intermediate host of COVID-19 remain unidentified (Li *et al.*, 2021).

Animals are integral to the emergence, transmission, and persistence of viruses, such as SARS-CoV-2 (Kuchipudi *et al.*, 2023). Bats, as natural reservoirs, contain several coronaviruses, while intermediary hosts including pangolins, camels, and civets enable cross-species transmission. Domesticated and wild animals can act as viral reservoirs, maintaining circulation and facilitating mutations that improve viral adaptability (Nandi, 2023). Comprehending ACE2 expression across species is crucial for finding prospective hosts, forecasting spillover events, and enhancing animal models for

investigating viral pathogenesis and therapeutic strategies (Silva and Nobre, 2024).

A protein (S-protein) spike that is present on the envelope acts as anchoring of SARS-CoV and expedites the virus penetration into host cells (Thirumugam *et al.*, 2023). The receptor-binding domain (RBD) of the S-protein of SARS-CoV primarily binds agonistin-converting enzyme 2 (ACE2), a host receptor. Interactions between two RBD residues (479 and 487) and four ACE2 residues (31, 35, 38, and 353) influence the dominant species barrier and the host susceptibility to SARS-CoV (Huang *et al.*, 2020). This suggests that COVID-19 has a higher capacity to transmit among people (Vardoulakis *et al.*, 2023). Angiotensin I converting enzyme 2 (ACE2) surface receptor was recognized for COVID-19 via contagion revisions using immortal human cells (HeLa cells) expressing angiotensin-converting enzyme-2 (ACE2) a type of protein isolated from several animals, including mankind, pork, rodents, shrews, and horseshoe bats (Bian and Li, 2021). Their discovery revealed that COVID-19 was capable of infecting all ACE2-expressing cells, except those from mice. The idea that ACE2 is the primary receptor for COVID-19 cell entry is further supported by this discovery. Prior studies have shown that the human ACE2 protein is present in several bodily organs (Beyerstedt *et al*., 2021). Using single-cell RNA sequencing, Zhao *et al*. (2020) recently found that type 2 alveolar epithelial cells in the lungs, together with superior and stratified arterial epithelial cells, significantly enhanced ACE2 expression.

For animal and veterinary inquiry, understanding ACE2 expression patterns in mammals is crucial because it clarifies the vulnerability of various species to SARS-CoV-2 and enhances our ability to model viral transmission dynamics (Ghosh and Malik, 2020). By identifying possible animal reservoirs or viral carriers, this study aids in the creation of effective preventive and control methods to protect the health of both animals and the general people (Dhama *et al.*, 2020). Furthermore, the results from these investigations can guide the veterinary care of impacted animals and deepen our understanding of viral interactions across species, ultimately contributing to a more comprehensive strategy for addressing zoonotic infections.

In light of the ongoing communal well-being crisis, it is important to discover efficacious antiviral medications to ensure human and animal health. Given the inherent confrontation of the mouse model to COVID-19, it is domineering to identify other animal models for medication testing and vaccine progress (Ng *et al.*, 2022). COVID-19 infects both humans and animals so developing an animal model to study this disease will be useful in public and veterinary health (Muñoz-Fontela *et al.*, 2020). Because of the vital function of angiotensin-converting enzyme-2 in pathological entrance, we investigated its evolutionary history, diversity, and pattern of dispersal in humans and many widely used physical representations (Zhao *et al.*, 2024). The consequences will deliver a critical understanding for choosing the optimal animal specimen for examination and therapy of COVID-19 in humans and animals (Tiwari *et al.*, 2020; Kuba *et al.*, 2021).

MATERIALS AND METHODS

Phylogenetic analysis: A phylogenetic tree was constructed using the protein polymers (amino acid) sequences of angiotensin-converting enzyme-2 recovered from 106 classes, including those belonging to the orders primate species, gnawers, placental mammals, ungulates, bats, and Carnivora. Utilizing MEGA X (v10.1.7), a consensus circular phylogenetic tree was constructed. Using the Jones-Taylor-Thornton (JTT) replacement model and 100 regeneration runs, the evolutionary tree was constructed using the optimum Likelihood criteria (Letunic and Bork, 2024). Utilizing the Adobe Illustrator CC (2018, v22.1), iTOL digital platform (https://itol.embl.de/), the tree was subsequently visualized and prepared.

Protein sequence alignment: Computational protein categorization orientations were conducted using ClustalW2. A final version of the figure was created for publishing using Adobe Illustrator CC (Hameduh *et al.*, 2020).

Visualization of ACE2 expression distribution: Data on humanoid countenance were attained from the GTEx (Genotype-Tissue Expression) catalog available at https://gtexportal.org/home/. The tissues identified by GTEx for analysis were: non-sun-exposed skin, the left ventricle of the heart, skeletal muscle, cortex of the brain, spleen, visceral omentum adipose tissue, thyroid, transverse colon, the hepatocyte, endocrine gland, pulmonary, adrenal gland. Subsequently, the models from the thorax, intestine (transverse), pulmonary, hepatocyte, cerebral, and adrenal glands underwent a systematic sequence of filtration processes. During sampling, we eliminated samples that satisfied the given procedures: transcriptome evidence that was not accurate, a RIN below 6.0, or a self-digestion notch over 2. The RIN (RNA integrity number) is a classic metric used to assess the superiority of RNA, while the autolysis score quantifies the degree of cellular or tissue damage caused by the organism's enzymes or processes. To mitigate the influence of sickness and medical intervention on ACE2 expression, only samples from persons who were in a state of good health before death were kept. Moreover, any therapeutic antiquities and reasons of expiry not previously stated were also omitted. The expression data for four more animals, from primates (macaques), carnivores (dogs), and rodents (rats and mice) were acquired from the diplomatic GEO database under the accession reference GSE125483. The expression pattern of ACE2 was represented by a boxplot, that was created by the ggplot2 package. After that, a significant difference was depicted between female and male groups, statistically, by using a t-test (Lean *et al.*, 2023).

ACE2 expression analysis in different organs: The wide-ranging pieces of evidence related to the pattern of gene expression were collected by the Human Protein Atlas database, it also has a role in investigating the ACE2 expression in different structures. Now it clarifies that we can understand the biological and pathological processes

of various cells, tissues, and even organs by analyzing the occurrences of ACE2 (Hikmet *et al.*, 2020).

ACE2 expression with related proteins: The expression of ACE2 and its related proteins was examined using a protein-protein interaction model generated by the STRING database. This platform helped to discover and map the protein's interaction network, which allowed for a comprehensive analysis of ACE2's functional interactions with other proteins (Wicik *et al.*, 2020). The study improved our understanding of ACE2's biological activities and potential pathways affected by these interactions by shedding the spotlight on the interactions between molecules in which it is engaged using STRING's large dataset.

RESULTS

Comparative analysis of ACE2 binding sites: A tree of about 106 species was designed for understanding the ACE2 protein sequences. Primates, rodents, placental mammals, ungulates, bats, and carnivores are among the species. The findings revealed that Rodents have a closer evolutionary relationship with Primates, but Carnivora, Artiodactyla, and Perissodactyla had a closer evolutionary relationship with Chiroptera. The genera Carnivora, Artiodactyla, and Perissodactyla have a closer evolutionary relationship with Chiroptera. The order Chiroptera includes bats that are widely recognized as the primary hosts for the majority of coronaviruses (Fig. 1). This phylogenetic analysis emphasizes the evolutionary closeness of Chiroptera (bats) to Carnivora, Artiodactyla, and Perissodactyla, indicating possible cross-species transmission risks and underscoring the role of bats as primary reservoirs for coronaviruses, which has important implications for zoonotic spillover events.

Certain animals from the Carnivora, Artiodactyla, and Perissodactyla groups are susceptible to coronavirus infections. This includes pigs and camels, which can serve as intermediate hosts for MERS-CoV. Additionally, species like the toddy cat, musang, and ferrets act as transitional hosts for SARS-CoV. We examined the amino acid variation at specific regions of ACE2 that are known to be essential for its binding attraction to COVID-19 and its ability to transmit across different species. The analysis of the sequence alignment showed that both the cynomolgus macaque and chimp possess the same sequence of protein polymers (amino acids) as humans at all five of these locations. This indicates that these species are very suitable for investigating SARS-CoV-2 infection.

Four similar sites and one distinct site (amino acid 82: M>T) are present in cattle and pigs. Ferrets, cats, and dogs each possess three similar protein sites and two distinct protein sites (amino acid 38: D>E, 82: M>T). Bats and mice have just two identical amino acids in common with humans, namely amino acids 38 and 353, and 35 and 38, respectively (Fig. 2). This investigation of amino acid variations underscores the potential for cross-species transmission of SARS-CoV-2, with animals like cynomolgus macaques and chimpanzees exhibiting significant resemblance to humans, rendering them optimal subjects for viral research. The existence of analogous or unique ACE2 binding sites in other species, including cattle and domestic animals, indicates differing susceptibilities to infection and highlights the necessity of identifying intermediary hosts in zoonotic transmission routes.

ACE2 expression in animal models: We analyzed ACE2 expression by examining existing data and gathering expression information from 706 humanoid tasters and 207 samples from four widely used animal models: crab-eating macaque, dog, mouse, and rat. Further study was conducted using data obtained from 12 tissues. To evaluate any variations in expression depending on sex, the samples were classified into masculine and feminine sets. Fig. 3 demonstrates that four specific tissues, counting the cardiac, intestinal, and thyroid, had elevated levels of ACE2 expression in humans. Furthermore, the expression of ACE2 showed considerable variation across the examined population in organs such as the colon, adipose tissue, and thyroid, indicating different levels of individual vulnerability to COVID-19 contamination.

The liver, lung, and thyroid of crab-eating macaques exhibited elevated levels of ACE2 expression. Dogs' epidermis, liver, and colon all showed high levels of ACE-2 expression, with the colons of male and female dogs exhibiting the most diversity in expression. The lung, adrenal gland, and epidermis of both mice and rats showed increased expression of ACE-2. Furthermore, the thyroid of rats and the colon of mice both showed increased ACE-2 expression. This thorough investigation of ACE2 expression demonstrates tissue-specific differences in people and animal models, emphasizing organs with heightened ACE2 levels, including cardiac, intestinal, and thyroid tissues in humans, and liver, lung, and thyroid in macaques. The identified sex-based disparities in ACE2 expression among species, notably in the canine colon and rat thyroid, imply possible variations in COVID-19 susceptibility, underscoring the necessity for customized strategies in both human and animal model investigations.

ACE2 expression analysis in different organs: CE2 expression was examined in a variety of organs as part of the bioinformatics assessment of possible animal models for SARS-CoV-2 to determine its distribution and significance for viral susceptibility. The information, which came from extensive gene expression databases, showed that ACE2 expression varied by organ, especially in organs that are important targets for SARS-CoV-2 infection, such as the heart, kidneys, gastrointestinal system, and lungs. These discoveries are essential for discovering animal models that faithfully replicate the organ-level manifestation of ACE2 seen in humans, which will enable more efficient research on viral entrance and disease **(**Fig. 4). The bioinformatics analysis of ACE2 expression in several organs highlights its essential role in SARS-CoV-2 vulnerability, especially in primary targets such as the heart, kidneys, gastrointestinal system, and lungs. These findings are crucial for discovering animal models that accurately replicate human ACE2 distribution, so enabling more precise investigations into viral entry mechanisms and disease etiology, ultimately improving the development of treatment options.

ACE2 expression with related proteins: ACE2 expression was analyzed in conjunction with several interacting proteins using a protein-protein interaction model constructed via the STRING database. The proteins identified as interacting with ACE2 include TMPRSS2, CLEC4M, ITGB1, KDM1A, HSPA5, GRM2, PDZK1, SLC6A19, SLC9A3R1, TGFBR2, SHANK1, SNX27, and biological processes, such as virus entry (TMPRSS2), immunological response (CLEC4M), cell binding (ITGB1), protein folding (HSPA5), and signaling networks (TGFBR2) (Fig. 5). The investigation of protein-protein interactions uncovers a complex network

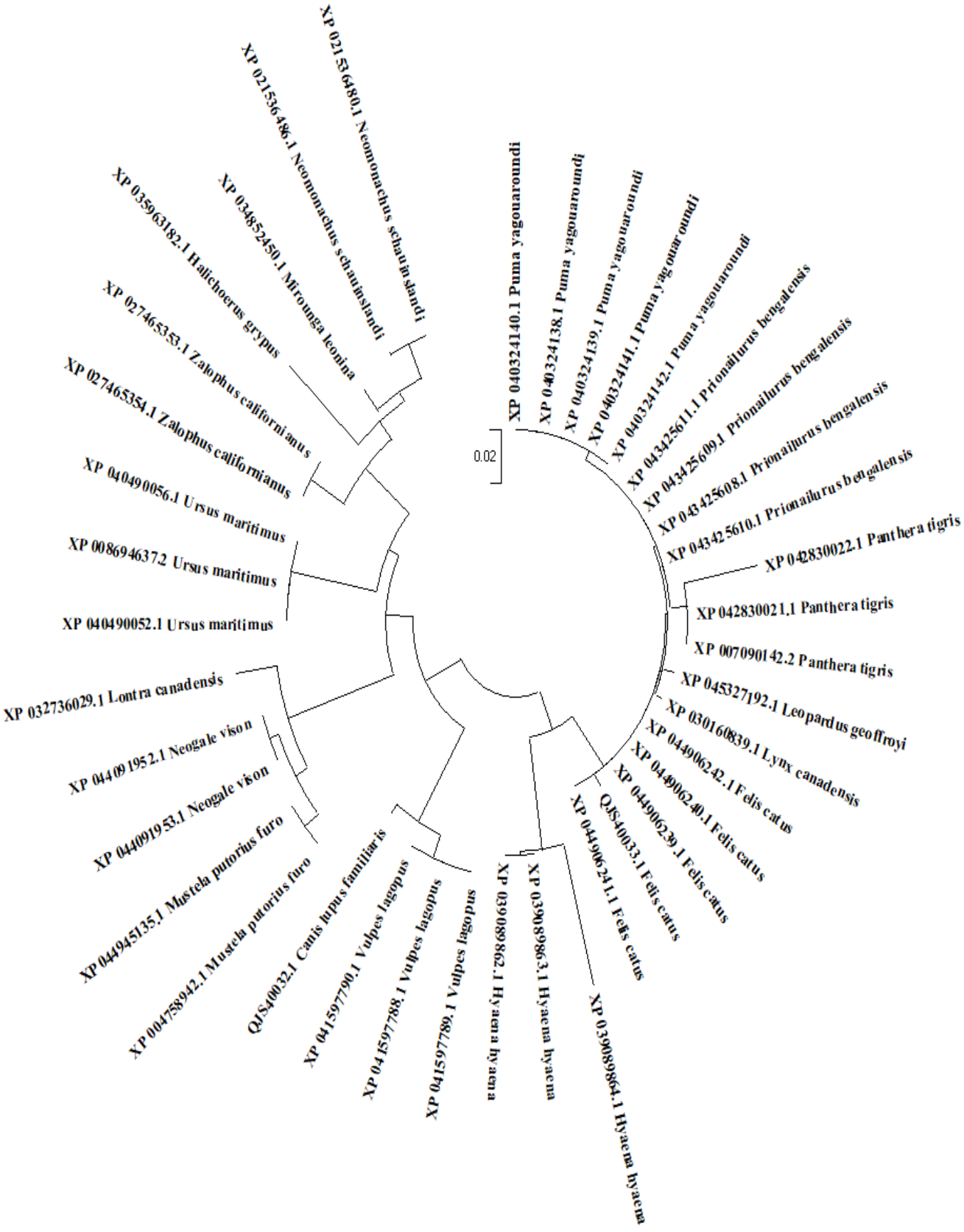

Fig. 1: The phylogenetic tree of various mammals constructed by maximum likelihood method.

Fig. 2: Alignment of five key hotspot residues of the ACE2 protein, highlighted in red. These amino acids are identified as critical for mediating interactions with the coronavirus, playing a pivotal role in viral binding and entry into host cells. Their positions and conservation across different species underscore their importance in the host-pathogen interface.

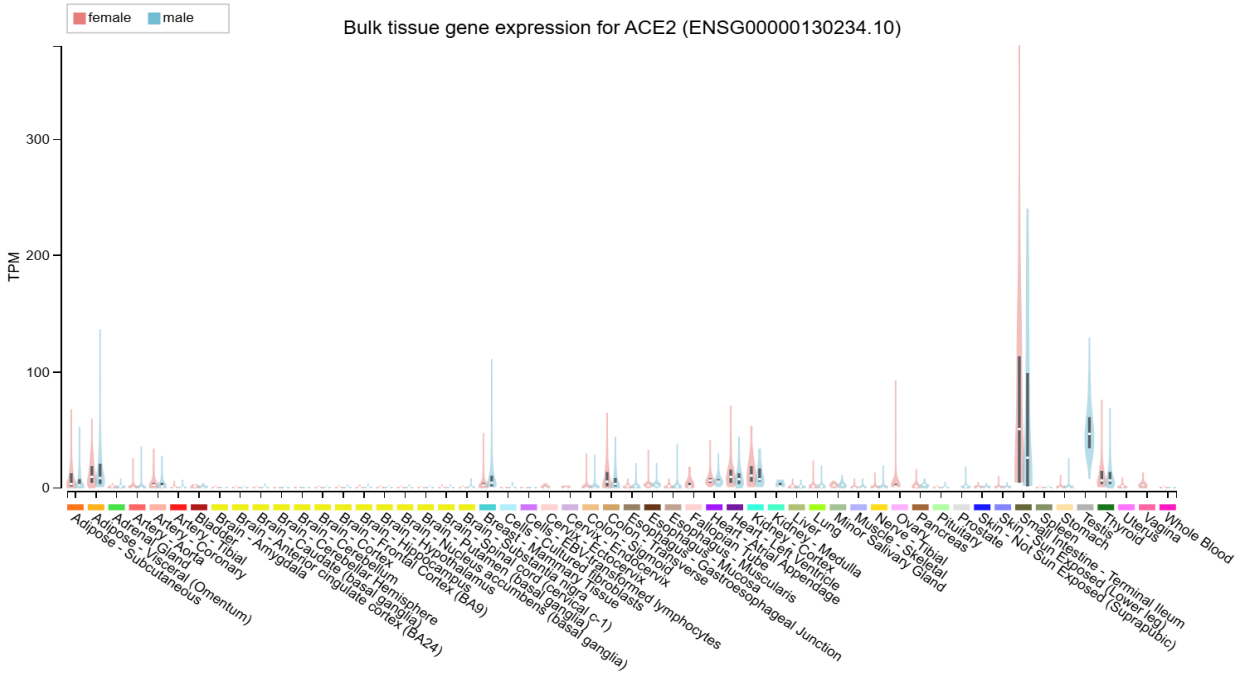

Fig. 3: Tissue-specific expression profile of ACE2 across different tissues in mammalian species. Expression levels are quantified using transcripts per kilobase million (TPM), providing a comparative overview of ACE2 distribution. The data highlight the varying expression patterns of ACE2, indicating its potential functional significance in different biological contexts.

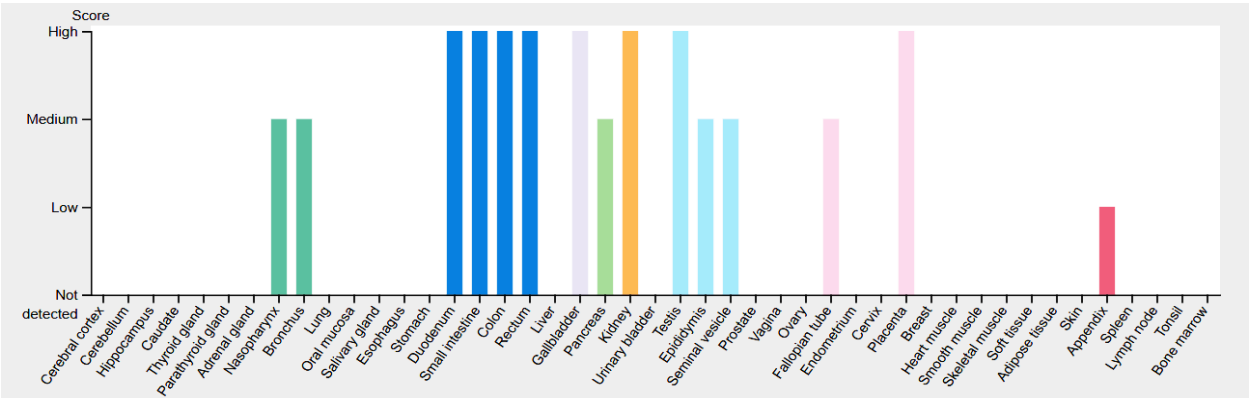

Fig. 4: ACE2 expression analysis in different organs.

of ACE2-associated proteins, underscoring its role in various physiological processes, such as viral entry, immunological response, cell adhesion, protein folding, and signaling. These interactions enhance our comprehension of ACE2's physiological responsibilities and indicate possible therapeutic targets for regulating ACE2-related pathways in SARS-CoV-2 infection and other illnesses.

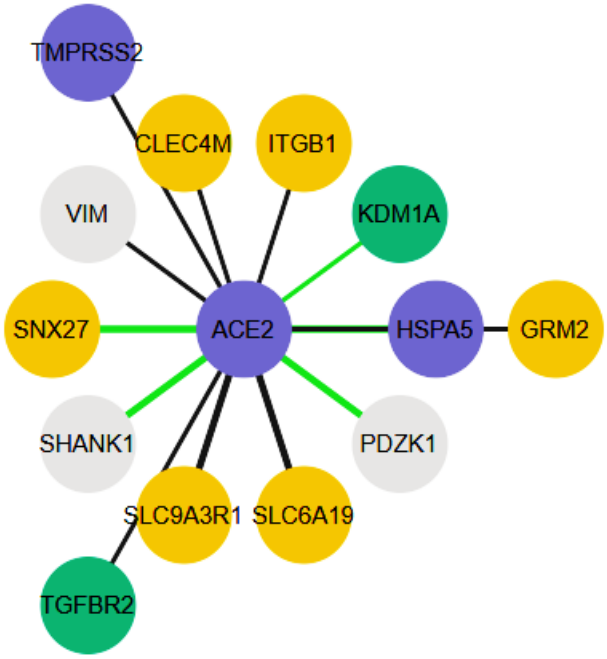

Fig. 5: ACE2 expression with related proteins.

DISCUSSION

The COVID-19 pandemic has had a significant impact on veterinary and public health across the world. The lack of completely successful treatment plans and antiviral treatments, despite tremendous efforts, emphasizes how urgently antiretroviral drugs and vaccines must be discovered, developed, and improved (Hu *et al.*, 2021). The intrinsic resistance of conventional mouse models to SARS-CoV-2 infection, which results from variations in angiotensin-converting enzyme 2 (ACE2) receptor affinity, has been a significant barrier in preclinical research. To manage this illness in humans and animals, it has become necessary to find the best biological model that closely resembles human physiological reactions to COVID-19 (Sharun *et al.*, 2020). For therapeutics and vaccine progress, a more reliable and understandable agenda will be availed through it (Lutz *et al.*, 2020).

Using bioinformatics methods, we looked at the ACE2 receptor's transcriptional patterns and historical preservation in different mammals. This work was crucial for selecting appropriate animal models for COVID-19 research. Surprisingly, we found that ACE2 expression is not uniform across tissues; rather, it is highly expressed in some tissues of both humans and animals, such as the thyroid, colon, heart, and fat. Some previous researchers also reported similar findings as observed in our study (Lazartigues *et al.*, 2020). The findings of Chen *et al.* (2024) and Havranek *et al.* (2023) also supported our findings. Based on these findings, it is clear that any animal model trying to replicate the human reaction to SARS must have identical ACE2 expression patterns, particularly in these critical organs.

In COVID-19 patients, symptoms including severe diarrhea, thyroiditis and myocarditis have been associated with elevated levels of ACE2 in the circulatory system and gastrointestinal tract (Bourgonje *et al.*, 2020). For example, it has been shown that the occurrence of these disorders is correlated with high levels of ACE2 expression in the gut and heart tissues. Moreover, similar to our observation a recent case study reported the development of subacute thyroiditis in a COVID-19 patient, prompting researchers to hypothesize that thyroid dysfunction could serve as an early indicator of COVID-19 infection (Yang *et al.*, 2024).

It was observed that ACE2 highly expressed in obese persons in adipose tissues which make them susceptible to SARS-CoV2. Sudhakar *et al.* (2022) found an increased susceptibility to SARS-CoV-2 among individuals with obesity, potentially linked to ACE2 expression levels in adipose tissue. Interestingly, their findings revealed no significant differences in ACE2 expression between males and females, suggesting the absence of a sex-based predisposition to infection. However, the higher infection rates observed in men may be attributed to increased exposure rather than biological differences in ACE2 receptor expression (Viveiros *et al.*, 2022; Sidhwani *et al.*, 2023).

In the context of preclinical research, our study underscores the importance of selecting an appropriate animal model that mirrors human ACE2 receptor distribution and function. Crucially, our analysis showed that the cynomolgus macaque's viral binding regions of ACE2 are the same as those in humans, highlighting the macaque's potential as the perfect model organism for COVID-19 study. Given its strong anatomical, hormonal, and physiological resemblance to humans and some other animals, such as cats and dogs, the cynomolgus macaque one of the most researched non-human primates emerges as a suitable model (Munshi *et al.*, 2021). Additionally, swabs from the nose, throat, anus, and plasma contained viral genomes, demonstrating the macaque's appropriateness as a model for researching the pathophysiology of COVID-19 and assessing possible treatments (Munster *et al.*, 2020). Because of their similarity to humans in terms of viral attachment sites and ACE2 gene expression, *Cynomolgus macaques* are a great model for studying SARS-CoV-2 infection in a preclinical setting (Johansen *et al.*, 2020). The fact that they can mimic the dynamics of human infections and are susceptible to the virus lends credence to their use as a model for learning more about COVID-19 and developing effective treatments.

Some animals are good models for studying viruses for example ferrets and cats, are susceptible to SARS-CoV-2 and exhibit very mild symptoms when infected (Muñoz-Fontela *et al.*, 2020). Transgenic mice that are engineered to generate human ACE2 receptors are a valuable model for studying the pathogenic pathways of SARS-CoV-2 (Winkler *et al.*, 2020). We used genomic techniques to investigate several features of the virus-host interface in response to the urgent need for an accurate animal model that accurately represents human responses

to SARS-CoV-2 and the inherent risks associated with infection trials. Some Similar studies were also conducted by some researchers in the past (Sun *et al.*, 2020). We focused on studying the viral binding affinity structural features of relevant proteins, evolutionary links across species, and the genetic manifestation of lethal receptors associated with SARS-CoV-2 infection. Yadav *et al.* (2021) also studied some of the relevant parameters observed in our study. The bioinformatics investigations provided valuable information about the molecular dynamics of the virus and its associations with other species, which helped in selecting suitable models for future research (Ma *et al.*, 2021).

A one-health approach that unites veterinary studies with human welfare activities is necessary to better understand these dynamics and to guide public health programs that seek to reduce the risks of infectious disease. By looking at ACE2 expression in several mammalian species, we were able to identify possible hosts of SARS-CoV-2 and highlight the importance of animal and human health in relation to zoonotic diseases. Islam and his colleges also conducted similar study understand the role of animals COVID-19 zoonosis. (Islam *et al.*, 2022). One health approach was also applied by Pepin and colleges for the surveillance of emerging viruses including corona virus for the assessment of human animal interface. This study lays the groundwork for future research into the elements that put both domesticated as well as wild animal populations in danger of viral transmission by detailing the diversity in receptor expression (Pepin *et al.*, 2021).

Conclusions: Identification of suitable animal models is pivotal for advancement of COVID-19 research in both veterinary and medical fields, particularly for the development of vaccines and antiviral therapies for humans and animals alike. Our bioinformatics analysis underscores the cynomolgus macaque as a compelling candidate, given its close physiological resemblance to humans and its ACE2 receptor compatibility with SARS-CoV-2. Furthermore, transgenic mice engineered to express human ACE2 receptors provide invaluable insights into viral pathogenesis, serving as a complementary model alongside primates in preclinical investigations. While bioinformatics offers a robust framework for model selection, in vivo validation remains essential to confirm their ability to accurately mimic human infection dynamics and therapeutic responses.

Authors contribution: AA, ZS and UI developed the idea, AA, ZS and UA wrote the manuscript, AA, ZS, KA and ZJ Proof read the manuscript, AA, TX and UI, Draw the illustrations, and ZJ helped in formatting the manuscript.

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REFERENCES

Beyerstedt S, Casaro EB and Rangel ÉB, 2021. COVID-19: angiotensin-converting enzyme 2 (ACE2) expression and tissue susceptibility to SARS-CoV-2 infection. Eur J Clin Microbiol Infect Dis 40:905-19.

Bian J and Li Z, 2021. Angiotensin-converting enzyme 2 (ACE2): SARS-CoV-2 receptor and RAS modulator. Acta Pharm Sin B 11:1–12.

Bourgonje AR, Abdulle AE, Timens W, *et al.*, 2020. Angiotensin-converting enzyme 2 (ACE2), SARS-CoV-2 and the pathophysiology of coronavirus disease 2019 (COVID-19). J Pathol 251:228–248.

Chen Z, Yuan Y, Hu Q, *et al.*, 2024. SARS-CoV-2 immunity in animal models. Cell Mol Immunol 21:119-33.

Dhama K, Patel SK, Sharun K, *et al.*, 2020. SARS-CoV-2: Jumping the species barrier, lessons from SARS and MERS, its zoonotic spillover, transmission to humans, preventive and control measures and recent developments to counter this pandemic virus. Travel Med Infect Dis 37:101830.

Ghosh S and Malik YS, 2020. Drawing comparisons between SARS-CoV-2 and the animal coronaviruses. Microorganisms 8:1840.

Gulati I, Khan S, Gulati G, *et al.*, 2023. SARS-CoV-2 origins: zoonotic Rhinolophus vs contemporary models. Biotechnol Genet Eng Rev 39:225-58.

Hameduh T, Haddad Y, Adam V, *et al.*, 2020. Homology modeling in the time of collective and artificial intelligence. Comput Struct Biotechnol J 18:3494-3506.

Havranek B, Lindsey GW, Higuchi Y, *et al.*, 2023. A computationally designed ACE2 decoy has broad efficacy against SARS-CoV-2 omicron variants and related viruses in vitro and in vivo. Commun Biol 6:513.

Hikmet F, Méar L, Edvinsson Å, *et al.*, 2020. The protein expression profile of ACE2 in human tissues. Mol Syst Biol 16:e9610.

Hu B, Guo H, Zhou P, *et al.*, 2021. Characteristics of SARS-CoV-2 and COVID-19. Nat Rev Microbiol 19:141-54.

Huang Y, Yang C, Xu X, *et al.*, 2020. Structural and functional properties of SARS-CoV-2 spike protein: potential antivirus drug development for COVID-19. Acta Pharmacol Sin 41:1141-49.

Islam A, Ferdous J, Islam S, *et al.*, 2022. Transmission dynamics and susceptibility patterns of SARS-CoV-2 in domestic, farmed and wild animals: Sustainable One Health surveillance for conservation and public health to prevent future epidemics and pandemics. Transbound Emerg Dis 69:2523-43.

Johansen MD, Irving A, Montagutelli X, *et al.*, 2020. Animal and translational models of SARS-CoV-2 infection and COVID-19. Mucosal Immunol 13:877-91.

Kaur N, Singh R, Dar Z, *et al.*, 2021. Genetic comparison among various coronavirus strains for the identification of potential vaccine targets of SARS-CoV2. Infect Genet Evol 89:104490.

Kuba K, Yamaguchi T and Penninger JM, 2021. Angiotensin-Converting Enzyme 2 (ACE2) in the pathogenesis of ARDS in COVID-19. Front Immunol 12:732690.

Kuchipudi S V, Tan C, van Dorp L, *et al.*, 2023. Coordinated surveillance is essential to monitor and mitigate the evolutionary impacts of SARS-CoV-2 spillover and circulation in animal hosts. Nat Ecol Evol 7:956-59.

Lazartigues E, Qadir MMF and Mauvais-Jarvis F, 2020. Endocrine significance of SARS-CoV-2's reliance on ACE2. Endocrinology 161:bqaa108.

Lean FZX, Cox R, Madslien K, *et al.*, 2023. Tissue distribution of angiotensin-converting enzyme 2 (ACE2) receptor in wild animals with a focus on artiodactyls, mustelids and phocids. One Health 16:100492.

Letunic I and Bork P, 2024. Interactive Tree of Life (iTOL) v6: recent updates to the phylogenetic tree display and annotation tool. Nucleic Acids Res gkae268.

Li L, Wang J, Ma X, *et al.*, 2021. A novel SARS-CoV-2 related coronavirus with complex recombination isolated from bats in Yunnan province, China. Emerg Microbes Infect 10:1683-90.

Lutz C, Maher L, Lee C, *et al.*, 2020. COVID-19 preclinical models: human angiotensin-converting enzyme 2 transgenic mice. Hum Genomics 14:1-11.

Ma L, Li H, Lan J, *et al.*, 2021. Comprehensive analyses of bioinformatics applications in the fight against COVID-19 pandemic. Comput Biol Chem 95:107599.

Muñoz-Fontela C, Dowling WE, Funnell SGP, *et al.*, 2020. Animal models for COVID-19. Nature 586:509-15.

Munshi I, Khandvilkar A, Chavan SM, *et al.*, 2021. An overview of preclinical animal models for SARS-CoV-2 pathogenicity. Indian J Med Res 153:17-25.

Munster VJ, Feldmann F, Williamson BN, *et al.*, 2020. Respiratory disease in rhesus macaques inoculated with SARS-CoV-2. Nature 585:268-72.

Nandi JS, 2023. Global Perspectives on the Transmission of Zoonotic RNA Viruses from Wild Animal Species to Humans: Zoonotic, Epizootic, and Anthropogenic Viral Pathogens. Elsevier.

Ng TI, Correia I, Seagal J, *et al.*, 2022. Antiviral drug discovery for the treatment of COVID-19 infections. Viruses 14:961.

Pepin KM, Miller RS and Wilber MQ, 2021. A framework for surveillance of emerging pathogens at the human-animal interface: pigs and coronaviruses as a case study. Prev Vet Med 188:105281.

Pereira ACE da S, Gallo LG, Oliveira AF de M, *et al.*, 2024. Costs of hospital admissions due to COVID-19 in the federal capital of Brazil: a study based on hospital admission authorizations. Brazilian J Infect Dis 28:103744.

Sharun K, Tiwari R, Patel SK, *et al.*, 2020. Coronavirus disease 2019 (COVID-19) in domestic animals and wildlife: advances and prospects in the development of animal models for vaccine and therapeutic research. Hum Vaccin Immunother 16:3043-54.

Sidhwani SK, Mirza T, Khatoon A, *et al.*, 2023. Angiotensin-converting enzyme 2 (ACE2) polymorphisms and susceptibility of severe SARS-CoV-2 in a subset of Pakistani population. Virol J 20:120.

Silva PV and Nobre CN, 2024. Computational methods in the analysis of SARS-CoV-2 in mammals: A systematic review of the literature. Comput Biol Med 108264.

Sudhakar M, Winfred SB, Meiyazhagan G, *et al.*, 2022. Mechanisms contributing to adverse outcomes of COVID-19 in obesity. Mol Cell Biochem 477:1155-93.

Sun S-H, Chen Q, Gu H-J, *et al.*, 2020. A mouse model of SARS-CoV-2 infection and pathogenesis. Cell Host Microbe 28:124-33.

Thirumugam G, Radhakrishnan Y, Ramamurthi S, *et al.*, 2023. A systematic review on impact of SARS-CoV-2 infection. Microbiol Res 271:127364.

Tiwari R, Dhama K, Sharun K, *et al.*, 2020. COVID-19: animals, veterinary and zoonotic links. Vet Q 40:169-82.

Vardoulakis S, Sheel M, Lal A, *et al.*, 2023. COVID-19 environmental transmission and preventive public health measures. Aust N Z J Public Health 44:333.

Viveiros A, Gheblawi M, Aujla PK, *et al.*, 2022. Sex-and age-specific regulation of ACE2: Insights into severe COVID-19 susceptibility. J Mol Cell Cardiol 164:13-16.

Wicik Z, Eyileten C, Jakubik D, *et al.*, 2020. ACE2 interaction networks in COVID-19: a physiological framework for prediction of outcome in patients with cardiovascular risk factors. J Clin Med 9:3743.

Winkler ES, Bailey AL, Kafai NM, *et al.*, 2020. SARS-CoV-2 infection of human ACE2-transgenic mice causes severe lung inflammation and impaired function. Nat Immunol 21:1327-35.

Yadav R, Chaudhary JK, Jain N, *et al.*, 2021. Role of structural and non-structural proteins and therapeutic targets of SARS-CoV-2 for COVID-19. Cells 10:821.

Yang S, Guan T, Yang H, *et al.*, 2024. Case report: Neglected subacute thyroiditis: a case following COVID-19 vaccination. Front Med 11:1349615.

Zhao J, Kang M, Wu H, *et al.*, 2024. Risk assessment of SARS-CoV-2 replicating and evolving in animals. Trends Microbiol 32:79-92.

Zhao Y, Zhao Z, Wang Y, *et al.*, 2020. Single-cell RNA expression profiling of ACE2, the receptor of SARS-CoV-2. Am J Respir Crit Care Med 202:756-59.