Human ACE2 Receptors Most Resemble those of Orangutans and Monkeys when Comparing Possible SARS-CoV-2 Intermediate Hosts

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Outline

- Several potential host species of SARS-CoV-2 exhibit changes in amino acid residues of ACE2 receptor
- ACE2 shows large percent sequence similarities across species, indicating their structural importance
- Of the five critical amino acids on the ACE2 receptor, those of orangutans and grivet monkeys most resembled humans

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Host Species Exhibit Changes in Critical Residues of ACE2 Receptor

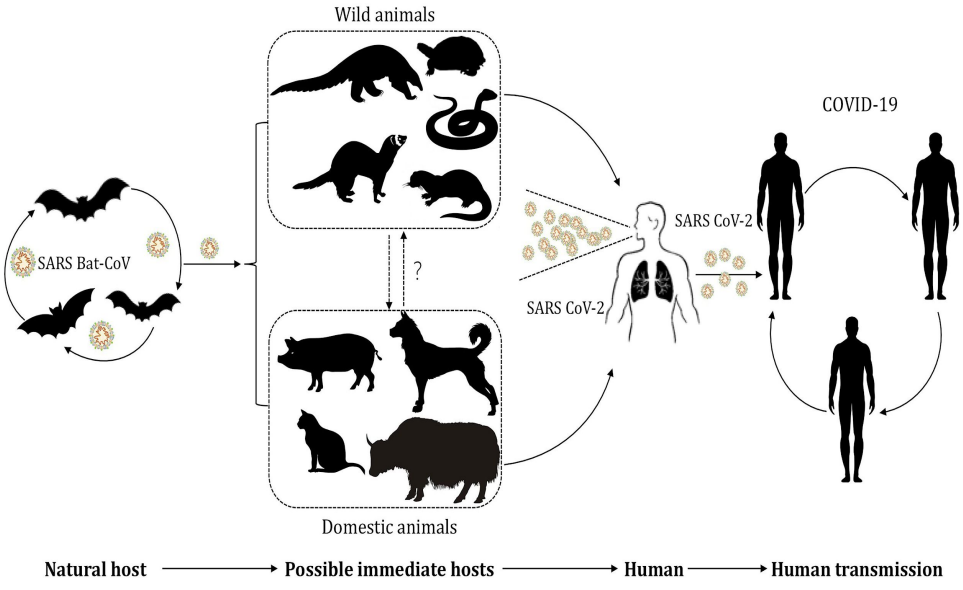
- Wan et al. 2020 explores differences between orthologous ACE2 sequences
  - Nine species compared to humans exhibit changes in critical amino acid residues
  - SARS-CoV-2 RBD observed binding to humans and bats, shows compatibility with civet ACE2

ACE2	31	35	38	82	353
Human	K	E	D	M	K
Civet	T	E	E	T	K
Bat	K	K	D	N	K
Mouse	N	E	D	S	H
Rat	K	E	D	N	H
Pig	K	E	D	T	K
Ferret	K	E	E	T	K
Cat	K	E	E	T	K
Orangutan	K	E	D	M	K
Monkey	K	E	D	M	K

(Wan et al., 2020)

Large Diversity of Species Sold in Wuhan Market

- SARS-CoV-2 traced back to Wuhan markets in 2019
- Besides bats, markets sell a large assortment of species to consumers
  - Includes mice, rats, pigs, ferrets, cats, orangutans, dromedary camels, squirrels, minks, turtles, snakes, chickens, foxes, and monkeys (Zhao et al., 2020)
- Potential intermediary hosts include
  - pangolins (Deng et al., 2020)
  - civets (Yuan et al., 2020)



(Zhao et al., 2020)

Research Question

Does comparing sequences of possible animal reservoirs noted by Wan et al. point towards one or more animal reservoirs as being closely related to humans, and therefore make them a good starting point for looking at SARS-CoV-2 lineage?

ACE2 Sequences of Potential Hosts Acquired

ACE2 Sequences

Based on potential hosts determined previously, eighteen species ACE2 sequences were located on NCBI database

Phylogenetic Tree

Generated using Phylogeny.fr to compare relationships of nineteen sequences and create a sequence alignment

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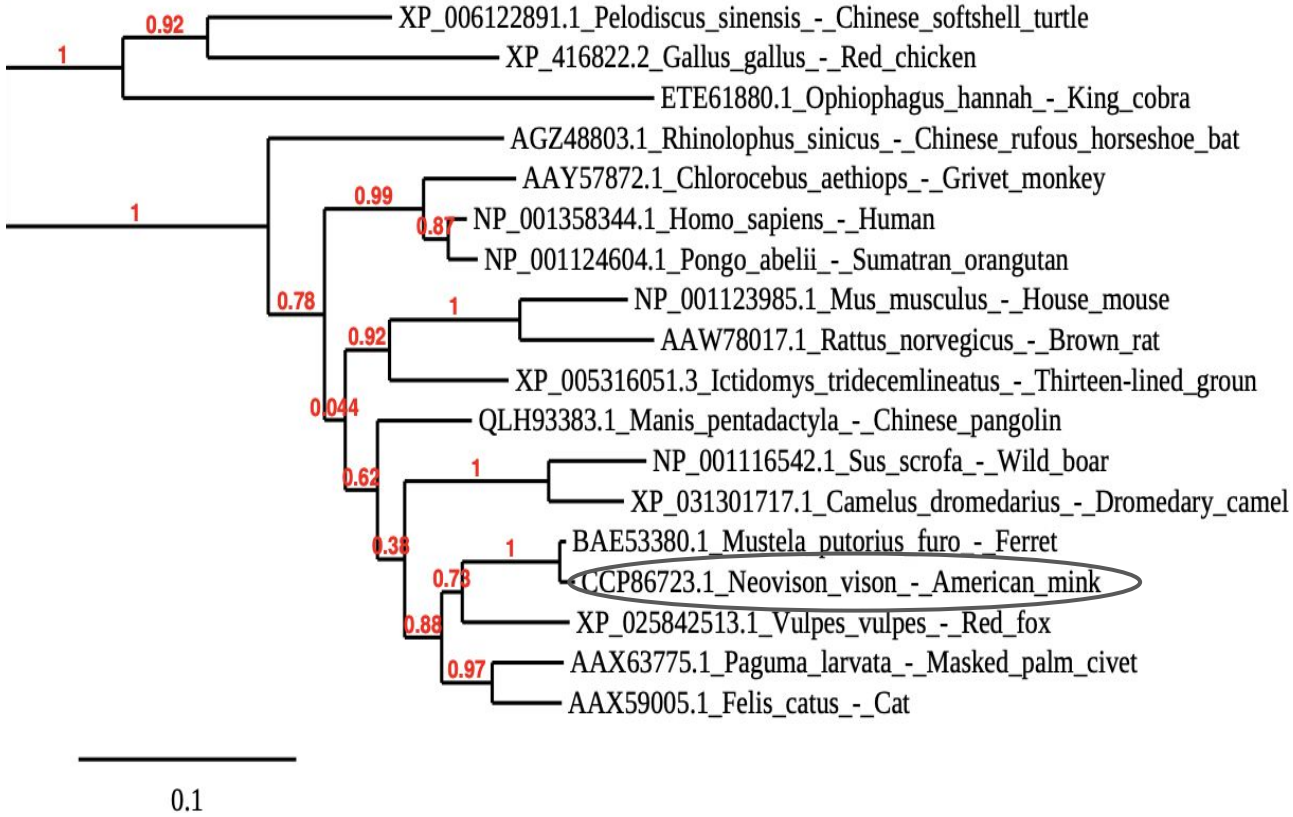
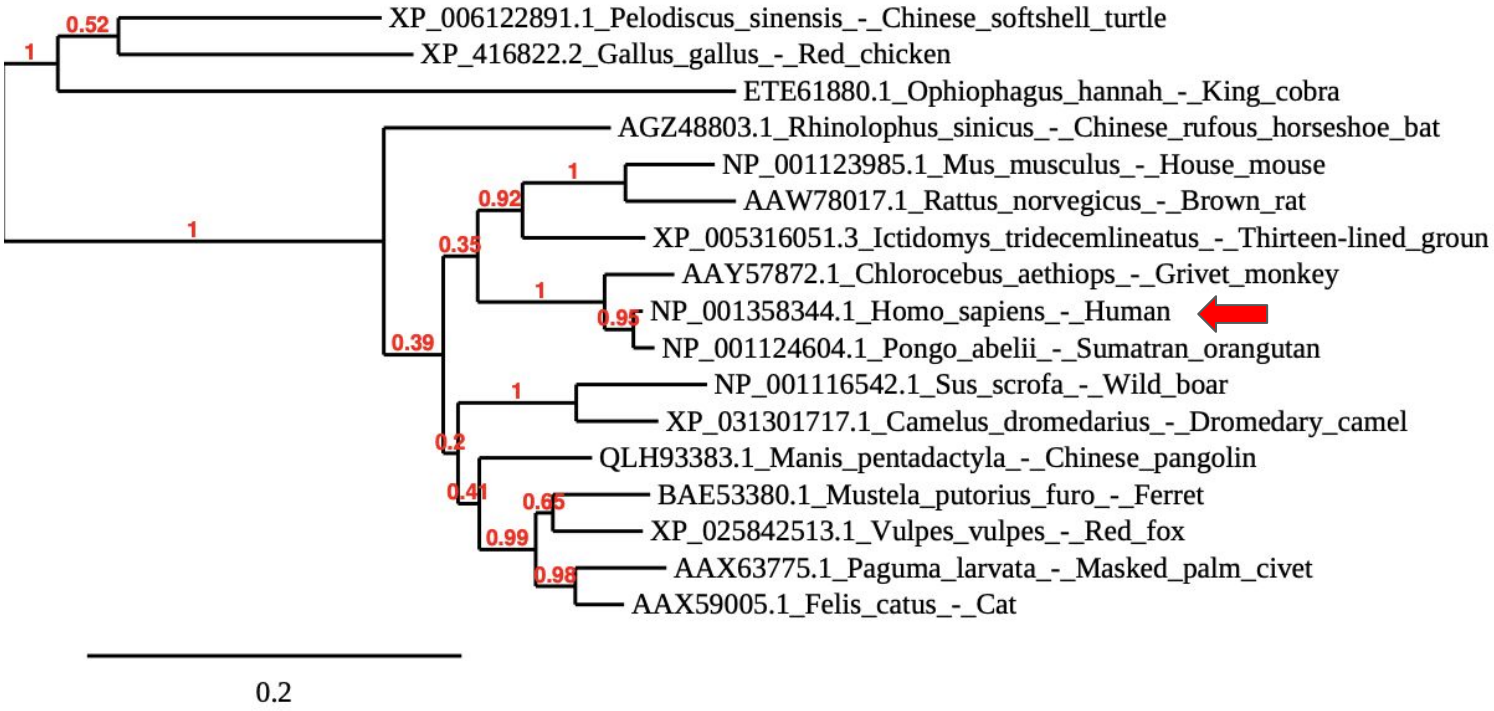
American Mink (*Neovison vison*) Only Available as Partial ACE2 Sequence



ACE2 Orthologues Show Closest Phylogenetic Relationship to Humans



ACE2 Percent Sequence Similarities Show Wide Range of Similarity to Humans

	King Cobra	Softshell Turtle	Chicken	Pig	Chinese Bat	Rat	Mouse	Civet	Pangolin	Ferret	Dromedary Camel	13 Lined Ground Squirrel	Domestic Cat	Fox	Grivet Monkey	Orangutan	Human
Human	77.9	85.5	87.4	93.9	94.0	94.2	94.2	94.3	94.4	94.4	95.0	95.0	95.4	95.4	98.4	99.8	100

- Animals commonly found in Wuhan market and orthologues show between 77.9% - 99.8% similarity to humans
  - Orangutans and monkeys most similar to humans
  - King cobras and softshell turtles show the least similarity

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Critical Residues of ACE2 Sequences were Identified by Wan et al. (2020)

Wan et al. (2020) identified the following residues of the civet ACE2 sequences for their comparison

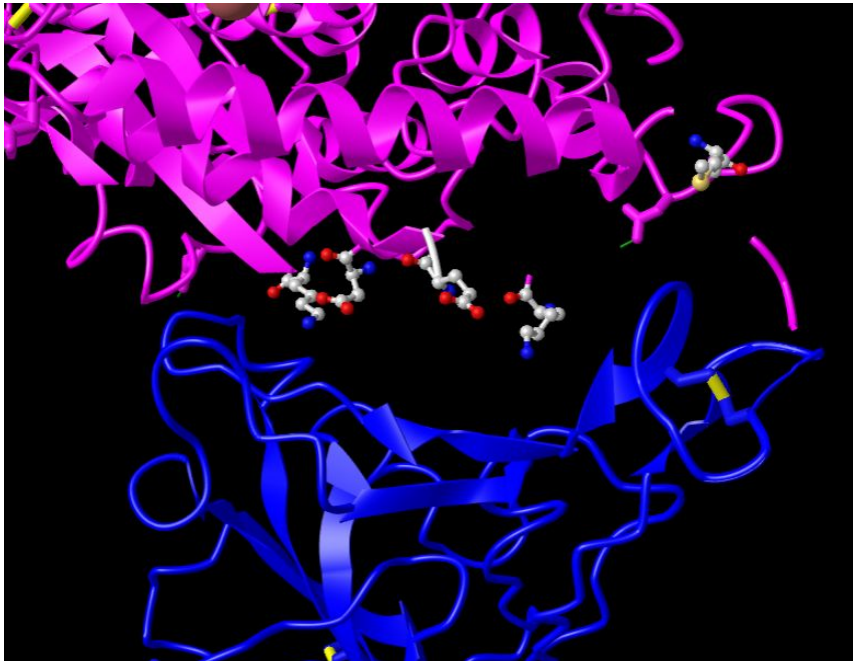
- T31
- E35
- E38
- T82
- K353

Visualization of Human Critical Residues was Made Using iCn3D Viewer

Here the human ACE2 is in pink and the SARS-CoV-2 spike protein in blue.

This was done by selecting the following residues & displaying them in the ball and stick style with atom colouring.

- K31
- E35
- D38
- M82
- K353



Comparison of Critical Residues Reveals Monkey & Orangutan ACE2 RBD most Similar to Human

	Chinese Bat	Pig	Dromedary Camel	Pangolin	Ferret	Fox	Civet	Domestic Cat	Mouse	Rat	13 Lined Ground Squirrel	Grivet Monkey	Human	Orangutan	King Cobra	Softshell Turtle	Chicken
31	K	K	E	K	K	K	T	K	N	K	K	K	K	K	Q	E	E
35	K	E	E	E	E	E	E	E	E	E	E	E	E	E	R	Q	R
38	D	D	D	E	E	E	E	E	D	D	D	D	D	D	D	D	D
82	N	T	T	N	T	T	T	T	S	N	A	M	M	M	M	K	R
353	K	K	K	K	K	K	K	K	H	H	K	K	K	K	K	K	K

Inability to Find Desired Sequences was a Possible Source of Error

- Chinese cobra ACE2 sequence unavailable
  - King cobra ACE2 used in substitute
- American mink (*Neovison vison*) only available as partial ACE2 sequence
  - Presented challenges in sequence alignment. Removed for clarity
- 13 lined ground squirrel was only available squirrel ACE2
  - Not found in China

Summary

- Orangutans and grivet monkeys are have the most similar ACE2 receptors to humans of the possible intermediate hosts
  - Known orthologues to humans
- Of the five critical amino acids that correspond to the RBD of SARS-CoV-2 on the ACE2 receptor, many were relatively conserved
  - Most organisms having between 2-3 of the 5 amino acids altered.
  - Two amino acid residues (35 and 353) show the greatest conservation
  - Residue 82 shows the least conservation of these residues

Future Research

- Understanding these potential intermediary hosts could inform a study looking to identify the lineage of SARS-CoV-2
- This would involve comparing the sequences of coronaviruses from these, and other, species to SARS-CoV-2
- Potential studies could identify which animals are able to act as intermediary hosts for future strains of SARS viruses that may evolve.

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