Insights into Cross-species Evolution of Novel Human Coronavirus SARS-CoV-2 and Defining Immune Determinants for Vaccine Development.

Arunachalam Ramaiah[1,*] and Vaithilingaraja Arumugaswami[2,*]

[1]Department of Ecology and Evolutionary Biology, University of California, Irvine, CA 92697, United States
[2]Department of Molecular and Medical Pharmacology, University of California, Los Angeles, CA 90095, United States

*Corresponding authors:
AR, aramaiah@uci.edu; VA, VArumugaswami@mednet.ucla.edu

ABSTRACT

Novel severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) outbreak in the city of Wuhan, China during December 2019, has now spread to various countries across the globe triggering a heightened containment effort. This human pathogen is a member of betacoronavirus genus carrying 30 kilobase of single positive-sense RNA genome. Understanding the evolution, zoonotic transmission, and source of this novel virus would help accelerating containment and prevention efforts. The present study reported detailed analysis of SARS-CoV-2 genome evolution and potential candidate peptides for vaccine development. This new coronavirus genotype might have been evolved from a bat-coronavirus by accumulating non-synonymous mutations, indels, and recombination events. Structural proteins Spike (S), and Membrane (M) had extensive mutational changes, whereas Envelope (E) and Nucleocapsid (N) proteins were very conserved suggesting differential selection pressures exerted on SARS-CoV-2 during evolution. Interestingly, SARS-CoV-2 Spike protein contains a 39 nucleotide sequence insertion relative to SARS-like bat-SL-CoVZC45/2017. Furthermore, we identified eight high binding affinity (HBA) CD4 T-cell epitopes in the S, E, M and N proteins, which can be commonly recognized by HLA-DR alleles of Asia and Asia-Pacific Region population. These immunodominant epitopes can be incorporated in universal subunit SARS-CoV-2 vaccine. Diverse HLA types and variations in the epitope binding affinity may contribute to the wide range of immunopathological outcomes of circulating virus in humans. Our findings emphasize the requirement for continuous surveillance of SARS-CoV-2 strains in live animal markets to better understand the viral adaptation to human host and to develop practical solutions to prevent the emergence of novel pathogenic SARS-CoV-2 strains.

Key words: SARS-CoV-2; Coronavirus 2; Evolution; Genome: Structural Proteins; Epitopes; Vaccine Target

INTRODUCTION

Current severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) outbreak in China and its subsequent spread to multiple countries across the continents precipitated a major global health crisis. Uncovering the SARS-CoV-2 genome evolution and immune determinants of its proteome would be crucial for outbreak containment and preventative efforts. Coronaviruses (CoVs) are single positive-stranded RNA viruses belonging to *Coronaviridae* family[1], which are divided into four genera: *Alpha-, Beta-, Delta- and Gamma-coronavirus*. Coronaviruses are commonly found in many species of animals, including bats, camels and humans. Occasionally, the animal coronaviruses can acquire genetic mutations by errors during genome replication or recombination mechanism, which can further expand their tropism to humans. The first human coronavirus was discovered in the mid-1960s. A total of six human coronavirus types were identified to be responsible for causing human respiratory ailments, which include two alpha coronaviruses (229E; NL63), and four beta CoVs (OC43; HKU1; SARS-CoV; MERS-CoV)[2]. Typically, these coronaviruses cause asymptomatic infection or severe acute respiratory illness, including fever, cough, and shortness of breath. However, other symptoms such as gastroenteritis and neurological diseases of varying severity have also been reported[3-5].

The recent two coronavirus human outbreaks were caused by betacoronaviruses, SARS-CoV (Severe Acute Respiratory Syndrome-CoV) and MERS-CoV (Middle East Respiratory Syndrome-CoV). In 2002, SARS-CoV outbreak was first reported in China, which spread globally causing hundreds of deaths with a 11% mortality rate. In 2012, MERS-CoV was first recognized in Saudi Arabia and subsequently in other countries, which has a fatality rate of 37%. Since December 2019, the seventh novel human betacoronavirus SARS-CoV-2 causing pneumonia outbreak was reported by the World Health Organization. This novel virus is linked with an outbreak of febrile respiratory illness in the city of Wuhan, Hubei Province, China with an epidemiological association to the Huanan Seafood Wholesale Market selling live farm and wild animals[6] and elsewhere in the city[1].

While the SARS-CoV-2 appears to be a zoonotic infectious disease, the specific animal species and other reservoirs need to be clearly defined. Identification of the source animal species for this outbreak would facilitate global public health authorities to inspect the trading route and the movement of wild and domestic animals to Wuhan and taking control measures to limit the spread of this disease[7]. The SARS-CoV-2 outbreak has a fatality rate of 2% with significant impact on global health and economy. As of 29 January 2020, the SARS-CoV-2 has spread to fifteen countries including Asia-Pacific region (Thailand, Japan, The Republic of Korea, Malaysia, Cambodia, Sri Lanka, Nepal, Vietnam, Singapore, and Australia), United Arab Emirates, France, Germany, Canada and the United States with 6065 confirmed cases, in which 5997 reported from China[8].

Here, we report a detailed genetic analysis of SARS-CoV-2 evolution based on 47 genome sequences available in Global Initiative on Sharing All Influenza Database (GISAID) (https://www.gisaid.org/) (Supplemental Table 1) and one genome sequence from GenBank. We further defined potential epitope targets in the structural proteins (SPs) for vaccine design.

MATERIALS AND METHODS

Phylogenetic tree construction. We employed the following methodologies for genetic analyses. The genome sequences of SARS-CoV-2 were obtained on 29 January 2020 along with genetically related 10 SARS-CoV/SARS-CoV-like virus genome sequences that we identified through the BLASTN search in NCBI database. We also included 10 sequences of MERS-CoV strains and one outgroup alphacoronavirus sequence. SARS-CoV-2 genome (30 Kb in size) codes for a large non-structural polyprotein ORF1ab that is proteolytically cleaved to generate 15/16 proteins, and four structural proteins - Spike (S), Envelope (E), Membrane (M) and Nucleocapsid (N), as well as five accessory proteins (ORF3a, ORF6, ORF7a, ORF8 and ORF10). The genome sequences were aligned using the MAFFTv7 program[9], which were exported to MEGA-X[10] for Neighbor-Joining (NJ) phylogenetic tree construction with 1000 bootstrap support and genetic distance calculations. Structural genes/proteins were aligned with closely related sequences using Clustal Omega Program[11].

High Binding Affinity (HBA) epitope identification. In order to identify the potential target peptides for vaccine design, the structural protein sequences of highly conserved and annotated representative SARS-CoV-2 strain Wuhan-Hu-1 (MN908947.3) were used to predict the CD4 T-cell epitopes (TCEs) with the Immune Epitope Database and Analysis Resource (IEDB) consensus tool by IEDB recommended prediction method[12,13]. To identify the most immunodominant (IMD) CD4 epitopes, we employed the computational approach described previously[14]. For all the possible 15-mer peptides from the SPs, the binding affinity was predicted using six most prevalent Human Leukocyte Antigen (HLA; MHC Class-II DR) alleles (http://allelefrequencies.net/) that cover most of the ethnic population in China (PRC), Thailand (TH) and Japan (JPN) (Supplemental Table 2). Since, the prevalence of dominant HLA-DR types may vary in human populations from different countries, the prevalent six HLA-DR alleles covering Asia-Pacific region[14] and Global population data sets have been used for binding affinity predictions to assess the generality of those predicted epitopes with high binding affinities (HBA). We calculated the average binding affinity score for each predicted 15-mer peptides against all the predominant HLA-DR types from each country/region using a sliding window approach (Figure 2). This approach is more efficient as the epitopes predicted with average HBA (IC <=10 nM), indicate that those epitopes are more likely to bind and enhance a cellular immune response in the studied population.

RESULTS AND DISCUSSION

Phylogenetic analysis reveals SARS-CoV-2 closely related to Bat coronavirus. Genome based phylogenetic tree analysis revealed a major cluster of large clade was formed with humans SARS-CoV-2 strains, SARS-CoV-like strains from bats and human SARS-CoV viruses (Figure 1A) with 100 bootstrap support. All SARS-CoV-2 strains were clustered together with 96% bootstrap support but separated from bat/bat-SARS like strains and human SARS-CoVs clusters, indicating that SARS-CoV-2 belongs to a novel genotype. A small cluster was formed by MERS-CoV strains, which was genetically unrelated to the strains circulating in the current outbreak. This result indicated that the causative agent of current outbreak is a betacoronavirus that is genetically and evolutionarily related to bat coronaviruses: bat/Yunnan/RaTG13/2013 and bat/SARS-CoV-like-viruses. Moreover, the result suggests that the evolutionary pressures exerted on SARS-CoV-2, as well as bat and human SARS-CoVs were different. Interestingly, strains from the current outbreak were clearly segregated from bat SARS-CoV-like viruses (isolated in 2015 and 2017), but located adjacent to the bat/Yunnan/RaTG13/2013 virus isolated in 2013. Besides, both SARS-CoV-2 and bat coronavirus clusters share a common ancestor (square), which is supported by 92% bootstrap replications in the phylogeny. The ancestors of both human SARS-CoV and SARS-CoV-2 share a common ancestor. Overall our data indicates that the SARS-CoV-2 is not closely clustering with either human SARS-CoV or bat-SARS-CoV like strains but shares a common ancestor.

The mean group genetic distance among SARS-CoV-2 strains was zero, indicating high genetic conservation among currently circulating viruses. Similar findings were observed in human SARS and MERS isolates, but bat SARS-like isolates showed a genetic distance of 0.04. The current circulating strains have close genetic associations with the 2013 bat coronavirus, bat/Yunnan/RaTG13/2013 (EPI-ISL-402131). The SARS-CoV-2 Wuhan-Hu-1 and bat/Yunnan/RaTG13/2013 strains share 96% sequence identity (genetic distance 0.04) with 1141 variable nucleotide substitutions and 48 INDELs (Figure 1C and D; Table 1A). While, bat-SL-CoVZC45/2017 virus and SARS human-BJ182-12/2008 virus from other clusters had 88% and 80% sequence identity (distance 0.23 and 0.46), 3542 and 5879 nucleotide substitutions and 137 and 535 INDELs, respectively with SARS-CoV-2 Wuhan-Hu-1, suggesting that bat/Yunnan/RaTG13/2013 strain is likely the most recent ancestor of currently circulating SARS-CoV-2 strains. This most recent ancestral coronavirus strain from bat species underwent significant evolution during 2013 to 2019 possibly by genetic shift before spreading to humans (Figure 1B). However, it is likely that coronaviruses from other animal species may also be a source for this outbreak. Comparison of Spike protein of SARS-CoV-2 and bat coronavirus bat/Yunnan/RaTG13/2013 showed 97.7% sequence identity with 29 non-synonymous changes and 4 amino acid insertions (position 681-684, PRRA) (Table 1C). Whereas, M protein shared 98.6% identity with one amino acid insertion (position 4, S) and N protein showed 99% identity with 4 non-synonymous changes (P37S, S215G, S243G, Q267A). Envelope protein shares 100% identity.

The genome alignment between the SARS-CoV-2 and bat (bat/Yunnan/RaTG13/2013) coronaviruses showed 2 of 7 insertions (Table 1A) in Spike gene, whereas the alignment of SARS-CoV-2 with distantly related SARS-like bat-SL-CoVZC45/2017 showed 9 of 15 insertions and 2 of 5 deletions (Figure 1C; Table 1B) in Spike gene. Interestingly, the Spike gene has a larger 39 nucleotide insertion (5'-aAT GGT GTT GAA GGT TTT AAT TGT TAC TTT CCT TTA CAA Tca-3') at genomic location 23022. Our alignment result suggests a possible occurrence of recombination and non-synonymous changes in Spike gene of SARS-CoV-2 which can alter the preference of viral attachment to host receptor. The non-synonymous changes in Nucleocapsid gene likely differentially regulate its binding to virus RNA genome and viral replication. Taken together, our findings implicate that overall genetic differences likely facilitated the novel coronavirus to adapt to humans from bat via altered cell receptor binding, changes in viral genome replication and better evading host immune detection.

Epitopes in SARS-CoV-2 structural proteins are differentially recognized by HLA-DR alleles. Host immune responses orchestrated by HLA molecules determine the outcome of infectious diseases. Antigen presentation by class II MHC isoforms play a critical role in inducing CD4+ Th1 and Th2 cell-mediated immune responses. It is important to understand the nature of immune selection pressure and evolution of immune escape viral mutants. Since the current outbreak is originated from Wuhan city of China, we set out to study the SARS-CoV-2 epitopes displayed by HLA-DR alleles predominantly present in the ethnic populations of China (PRC), Thailand (TH), Japan (JPN) and Asia-Pacific Region (APR). Thus, we calculated the average binding affinity score of each of the predicted 15-mer peptides from functionally important four structural proteins of SARS-CoV-2.

A total of 134 unique average HBA TCEs were identified in 16 islands/hotspots across the structural protein sequences (Figure 2; Supplemental Table 2). The lowest and highest numbers of average HBA TCEs were identified in nucleocapsid protein (n=11), and spike protein (n=78), respectively. This result reveals that mutations in spike protein may contribute to changes in viral virulence observed in SARS-CoV-2. The overall binding affinity patterns of HLA-DR alleles from different countries were similar, however there are some exceptions: i) no HBA epitope was identified in nucleocapsid protein for TH, JPN and APR dominant HLA-DR allele, while this protein exhibited 3 and 8 TCEs for PRC and Global population set alleles, and ii) HLA-DR alleles for APR and TH recognized lower (n=15) and higher (n=65) number of HBA epitopes, respectively. Nevertheless, delineating common epitopes is crucial for designing universally potent subunit vaccine. Thus, we investigated common epitopes that are recognized by all dominant HLA-DR alleles prevalent in the five ethnic populations. Comprehensively, 8 unique HBA epitopes distributed across Spike (n=2; 232-GINITRFQTLLALHR-246; and 233-INITRFQTLLALHRS-247), Envelope (n=3; 55-SFYVYSRVKNLNSSR-69; 56-FYVYSRVKNLNSSRV-70; and 57-YVYSRVKNLNSSRVP-71) and Membrane (n=3; 97-IASFRLFARTRSMWS-111; 98-ASFRLFARTRSMWSF-112; and 99-SFRLFARTRSMWSFN-113) proteins, were identified (Figure 2; Supplemental Table 2). The high affinity epitopes identified in the functionally important regions of Spike and Membrane proteins could be critical in modulating immune responses to circulating SARS-CoV-2. Many of the low binding affinity regions in the structural proteins may favor viruses to evade host antiviral immunity. Our analysis suggests that a subunit vaccine containing the eight immunodominant HLA-DR epitopes possibly induce effective antiviral T-cell and antibody responses in different ethnic populations.

In summary, the present study provided a detailed genetic analysis of SARS-CoV-2 genome evolution and potential universal epitopes for subunit vaccine development. This SARS-CoV-2 strain might be evolved from the bat-coronavirus by accumulating favorable genetic changes for human infection.

ACKNOWLEDGEMENTS

We gratefully acknowledge the authors, the originating and submitting Laboratories for their sequence and metadata shared through GISAID and GenBank, on which this research is based.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

REFERENCES

1. Huang et al. 2020. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. *The Lancet* (2020) DOI:https://doi.org/10.1016/S0140-6736(20)30183-5

2. CDC. Accessed 26 January 2020. (2020) https://www.cdc.gov/coronavirus/types.html.

3. Wang, L.F. et al. Review of bats and SARS. *Emerg. Infect. Dis.* **12**, 1834-1840 (2006).

4. Ge, X.Y. et al. Isolation and characterization of a bat SARS-like coronavirus that uses the ACE2 receptor. *Nature* **503**, 535-538 (2013).

5. Chen, Y. & Guo, D. Molecular mechanisms of coronavirus RNA capping and methylation. *Virol. Sin*. **31**, 3-11 (2016).

6. WHO. Emergencies preparedness, response. Pneumonia of unknown origin – China WHO. Disease outbreak news (2020). 5 January, Accessed 12 Jan 2020.

(2020a.) Available at: https://www.who.int/csr/don/05-january-2020-pneumonia-of-unkown-cause-china/en/

7. Hui, D. S. et al. 2020. The continuing 2019-nCoV epidemic threat of novel coronaviruses to global health — The latest 2019 novel coronavirus outbreak in Wuhan, China. *Int. J. Infect. Dis.* 91, 264-266 (2020).

8. WHO. The Situation Report - 9. 29 January 2020. (2020b) Available at: https://www.who.int/docs/default-source/coronaviruse/situation-reports/20200129-sitrep-9-ncov.pdf?sfvrsn=9e51db96_2

9. Katoh, K. et al. MAFFT online service: multiple sequence alignment, interactive sequence choice and visualization. *Brief. Bioinform.* **20**, 1160-1166 (2019).

10. Kumar, S. et al. MEGA X: Molecular Evolutionary Genetics Analysis across computing platforms. *Mol. Biol. Evol.* **35**, 1547-1549 (2018).

11. Sievers, F. et al. Fast, scalable generation of high-quality protein multiple sequence alignments using Clustal Omega. *Mol. Syst. Biol*. **7**, 539 (2011).

12. Wang, P. et al. A systematic assessment of MHC class II peptide binding predictions and evaluation of a consensus approach. *PLoS Comput. Biol*. **4**, e1000048 (2008).

13. Vita, R. et al. 2014. The immune epitope database (IEDB) 3.0. *Nucl. Acids Res.* **43**, D405–D412 (2014).

14. Ramaiah, A. et al. Complexity of type-specific 56 kDa antigen CD4 T-cell epitopes of *Orientia tsutsugamushi* strains causing scrub typhus in India. *PLoS ONE* **13**, e0196240 (2018).

FIGURE LEGENDS

Figure 1 - Phylogenetic relationships of novel SARS-CoV-2 (*2019-nCoV*) viral strains and the possible route of viral evolution and disease transmission. (A) NJ bootstrap consensus tree was reconstructed based on the genome sequences with 1000 bootstrap replications. SARS-CoV-2 strains (n=48 including one bat strain) from current outbreak, SARS-like-CoV (n=2), SARS-CoV (n=8) and MERS-CoV (n=10) viruses are colored in red, orange, yellow and green, respectively. The alphacoronavirus (MN056942) was used as an outgroup control (blue). (B) Evolution of a SARS-CoV-2 virus from bat/unknown animals to humans and spread among humans. (C) Dot plots comparison between the human SARS-CoV-2 (MN908947.3) and bat/Yunnan/RaTG13/2013 (EPI-ISL-402131) (left), or bat SARS-CoV-like (MG772933) viruses (middle); or human SARS-CoV (EU371564) (right). All these viruses were reported from China. (D) The proportion of conserved, variable (Single Nucleotide Polymorphism) and INDELs in SARS-CoV-2 was uncovered by aligning it to bat-coronavirus (left), bat SARS-CoV-like (middle) and human SARS-CoV (right).

Figure 2 - Comparison of SARS-CoV-2 structural proteins' epitopes with mean HBA to predominant HLA-DR alleles from China (PRC), Thailand (TH), Japan (JPN), Asia-Pacific region (APR) and Global population. The mean binding affinity score was calculated from the binding affinity (score) of each predicted 15-mer HBA peptides (sliding window approach) from four structural proteins with six predominant HLA-DR types from China (PRC), Thailand (TH), Japan (JPN), Asia-Pacific region (APR) and Global population sets. Functional islands with mean HBA TCEs (IC <= 10nM) in the structural proteins were located in the horizontal orange color. The epitopes having mean HBA to all five population HLA-DR alleles are highlighted with vertical orange bars.

Table 1 - Details of the insertions and deletions (INDELs) in the genome of SARS-CoV-2 (MN908947.3) relative to **A)** bat/Yunnan/RaTG13/2013 (GISAID #EPI_ISL_402131) and **B)** bat-SL-CoVZC45/2017 (MG772933.1) viruses. **C)** List of non-synonymous changes and INDELs in the four structural proteins of SARS-CoV-2 relative to bat/Yunnan/RaTG13/2013.

Table 1A.

Coding-sequence	Starting coordinate in the alignment	Ending coordinate in the alignment	# Insertion	Insertion (coordinates)	# Deletion	Deletion (coordinates)
5' UTR	1	264	1	1-15(attaaaggtttatac)	0	
ORF1	266	21555	1	3331-3333(TAT)	0	
S	21563	25384	1	23601-23612(ctcctcggcggg)	0	
ORF3	25393	26220	0		0	
E	26245	26472	0		0	
M	26523	27191	1	26532-26535(tcc)	0	
ORF6	27202	27387	1	27386(A)	0	
ORF7	27394	27759	0		0	
ORF8	27894	28259	0		0	
N	28274	29533	0		0	
ORF10	29558	29674	0		0	
3' UTR	29675	29903	2	298835(C), 29891-29903(aaaaaaaaaaaaa)	0	

Table 1B.

Coding-sequence	Starting coordinate in the alignment	Ending coordinate in the alignment	# Insertion	Insertion (coordinates)	# Deletion	Deletion (coordinates)
5' UTR	1	265	1	135(A)	0	
ORF1	266	21564	3	3046-3048 (AGA), 3224-3229 (CAAACT), 3265-3276 (AACTACTATTCA)	3	3163-3165(TTT), 3328-3330(TAC), 3953-3955(GCT)
S	21574	25402	9	21727 (T), 21779-21787 (CATGTCTCT), 22019-22021 (AAA), 22340-22348 (TCTTCTTCA), 22902-22910 (TTGATTCTA), 22950-22955 (GGAAGT), 22993-22995 (AAT), 23022-23060 (ATGGTGTTGAAGGTTTTAATTGTTACTTTCCTTTACAAT), 23619-23630 (CTCCTCGGCGGG)	2	21708(D), 21803-21908(GCCACA)
ORF3	25411	26238	0		0	
E	26263	26490	0		0	
M	26541	27209	0		0	
ORF6	27220	27405	0		0	
ORF7	27412	27777	0		0	
ORF8	27912	28277	0		0	
N	28292	29551	0		0	
ORF10	29576	29692	0		0	
3' UTR	29693	29921	2	29876-29877 (TT), 29918-29921 (AAAA)	0	

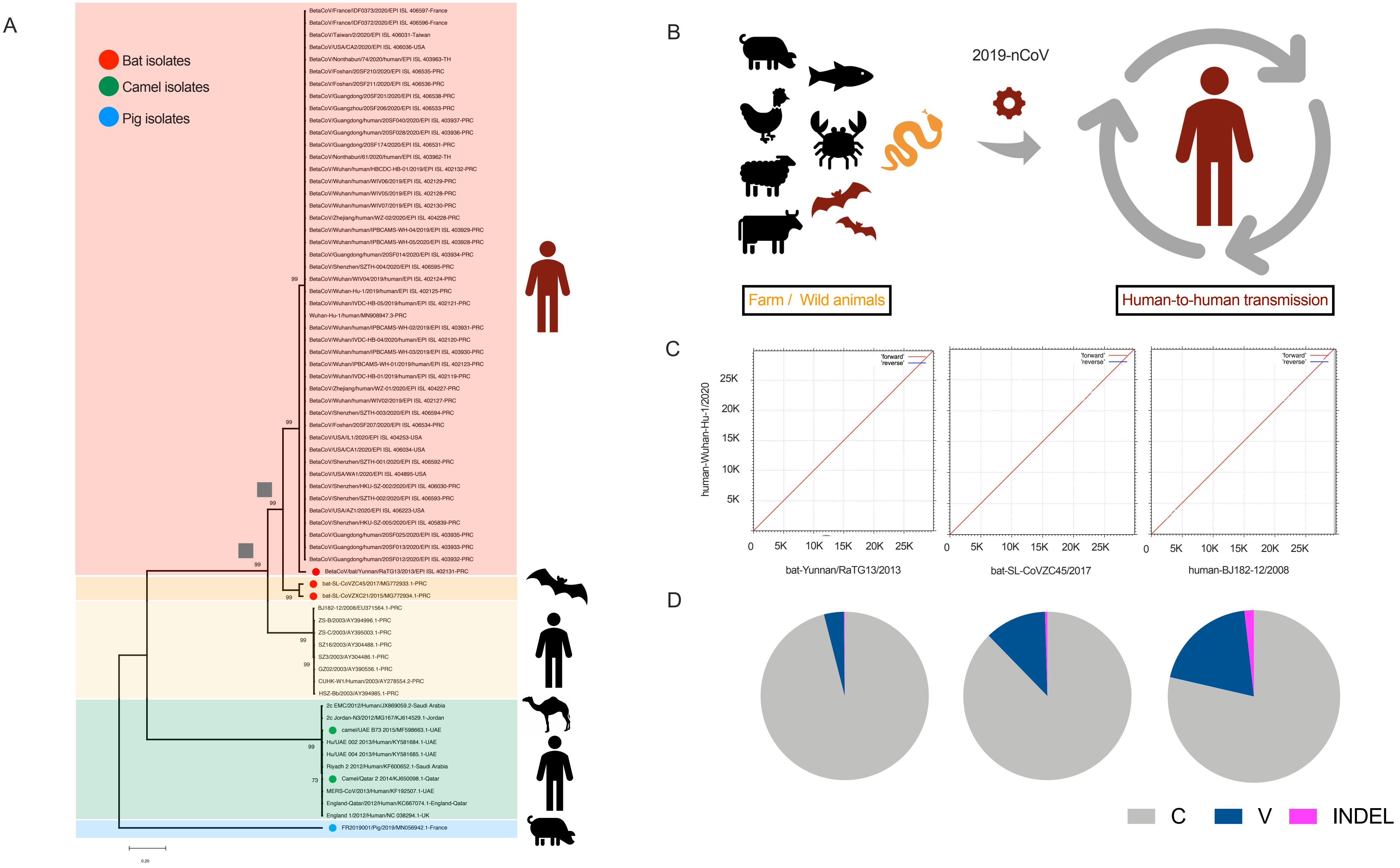
Table 1C.

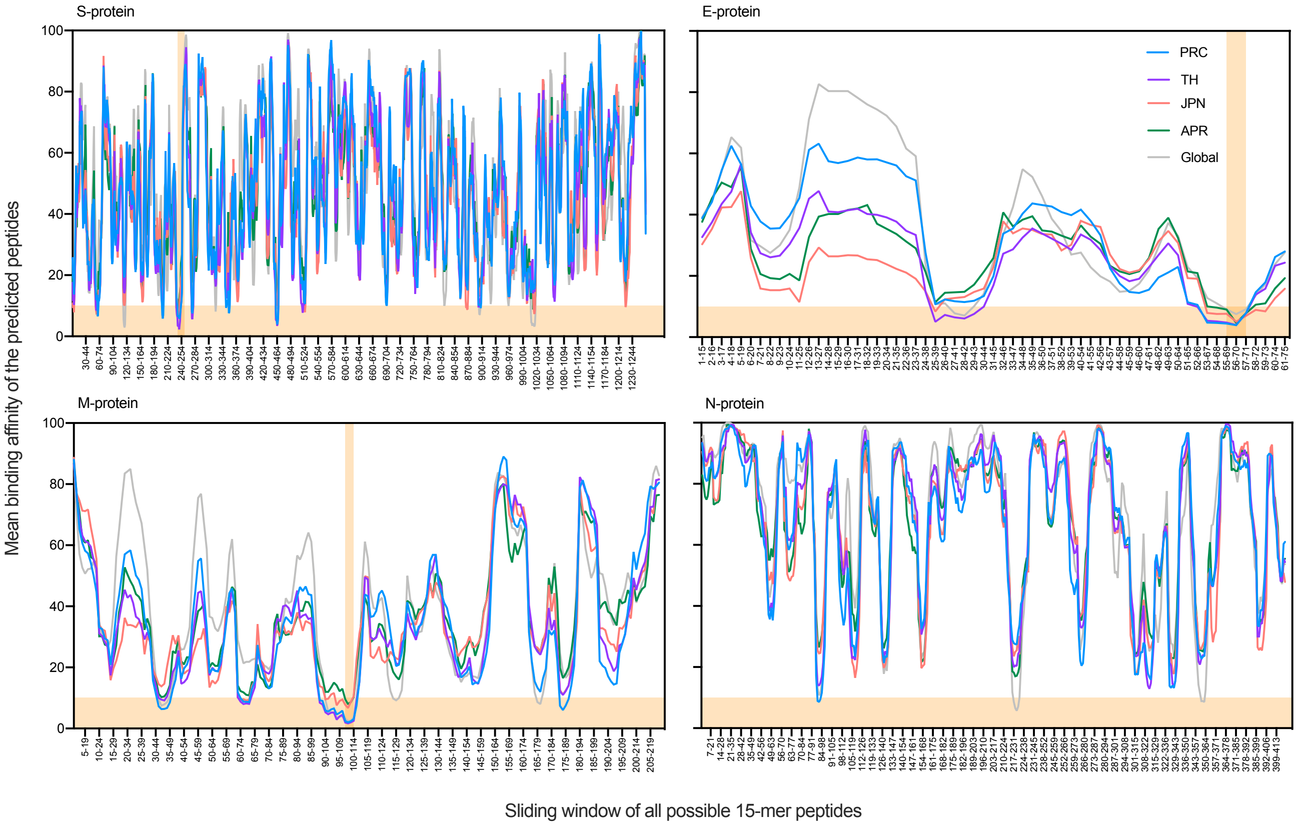
Protein	**Non-synonymous / amino acid change**	**Insertion position and amino acids**
S	S32F, L50S, I76T, P218Q, D324E, T346R, T372A, T403R, K439N, H440N, I441L, A443S, E445V, F449Y, A459S, K478T, Q483V, T484E, L486F, Y490F, Y493Q, R494S, Y498Q, D501N, H505Y, N519H, A604T, S1125N, I1228V	681-684,PRRA
E	nil	nil
M	nil	4,S
N	P37S, S215G, S243G, Q267A	nil

SUPPLEMENTAL TABLES

Supplemental Table 1 - Summary of authors, source laboratories and metadata from GISAID for SARS-CoV-2 genomes analyzed in this study.

Supplemental Table 2 - Amino acid sequences of high confidence mean HBA (IC <= 10nM) CD4 T-cell epitopes from functionally important regions of the four structural proteins (S, E, M and N) of SARS-CoV-2. The details of protein name, starting and ending positions of epitopes in the given protein, and epitope sequences are provided. The unique epitope sequences for each protein and the universal epitopes commonly recognized by HLA-DR alleles of all five population sets (highlighted in red) are listed at the end of each table.


A
Bat isolates
Camel isolates
Pig isolates
BetaCoV/France/IDF0373/2020/EPI ISL 406597-France
BetaCoV/France/IDF0372/2020/EPI ISL 406596-France
BetaCoV/Taiwan/2/2020/EPI ISL 406031-Taiwan
BetaCoV/USA/CA2/2020/EPI ISL 406036-USA
BetaCoV/Nonthaburi/74/2020/human/EPI ISL 403963-TH
BetaCoV/Foshan/20SF210/2020/EPI ISL 406535-PRC
BetaCoV/Foshan/20SF211/2020/EPI ISL 406536-PRC
BetaCoV/Guangdong/20SF201/2020/EPI ISL 406538-PRC
BetaCoV/Guangzhou/20SF206/2020/EPI ISL 406533-PRC
BetaCoV/Guangdong/human/20SF040/2020/EPI ISL 403937-PRC
BetaCoV/Guangdong/human/20SF028/2020/EPI ISL 403936-PRC
BetaCoV/Guangdong/20SF174/2020/EPI ISL 406531-PRC
BetaCoV/Nonthaburi/61/2020/human/EPI ISL 403962-TH
BetaCoV/Wuhan/human/HBCDC-HB-01/2019/EPI ISL 402132-PRC
BetaCoV/Wuhan/human/WIV06/2019/EPI ISL 402129-PRC
BetaCoV/Wuhan/human/WIV05/2019/EPI ISL 402128-PRC
BetaCoV/Wuhan/human/WIV07/2019/EPI ISL 402130-PRC
BetaCoV/Zhejiang/human/WZ-02/2020/EPI ISL 404228-PRC
BetaCoV/Wuhan/human/IPBCAMS-WH-04/2019/EPI ISL 403929-PRC
BetaCoV/Wuhan/human/IPBCAMS-WH-05/2020/EPI ISL 403928-PRC
BetaCoV/Guangdong/human/20SF014/2020/EPI ISL 403934-PRC
BetaCoV/Shenzhen/SZTH-004/2020/EPI ISL 406595-PRC
99
BetaCoV/Wuhan/WIV04/2019/human/EPI ISL 402124-PRC
BetaCoV/Wuhan-Hu-1/2019/human/EPI ISL 402125-PRC
BetaCoV/Wuhan/IVDC-HB-05/2019/human/EPI ISL 402121-PRC
Wuhan-Hu-1/human/MN908947.3-PRC
BetaCoV/Wuhan/human/IPBCAMS-WH-02/2019/EPI ISL 403931-PRC
BetaCoV/Wuhan/IVDC-HB-04/2020/human/EPI ISL 402120-PRC
BetaCoV/Wuhan/human/IPBCAMS-WH-03/2019/EPI ISL 403930-PRC
BetaCoV/Wuhan/IPBCAMS-WH-01/2019/human/EPI ISL 402123-PRC
BetaCoV/Wuhan/IVDC-HB-01/2019/human/EPI ISL 402119-PRC
BetaCoV/Zhejiang/human/WZ-01/2020/EPI ISL 404227-PRC
BetaCoV/Wuhan/human/WIV02/2019/EPI ISL 402127-PRC
BetaCoV/Shenzhen/SZTH-003/2020/EPI ISL 406594-PRC
99
BetaCoV/Foshan/20SF207/2020/EPI ISL 406534-PRC
BetaCoV/USA/IL1/2020/EPI ISL 404253-USA
BetaCoV/USA/CA1/2020/EPI ISL 406034-USA
BetaCoV/Shenzhen/SZTH-001/2020/EPI ISL 406592-PRC
BetaCoV/USA/WA1/2020/EPI ISL 404895-USA
BetaCoV/Shenzhen/HKU-SZ-002/2020/EPI ISL 406030-PRC
BetaCoV/Shenzhen/SZTH-002/2020/EPI ISL 406593-PRC
99
BetaCoV/USA/AZ1/2020/EPI ISL 406223-USA
BetaCoV/Shenzhen/HKU-SZ-005/2020/EPI ISL 405839-PRC
BetaCoV/Guangdong/human/20SF025/2020/EPI ISL 403935-PRC
BetaCoV/Guangdong/human/20SF013/2020/EPI ISL 403933-PRC
BetaCoV/Guangdong/human/20SF012/2020/EPI ISL 403932-PRC
99
BetaCoV/bat/Yunnan/RaTG13/2013/EPI ISL 402131-PRC
bat-SL-CoVZC45/2017/MG772933.1-PRC
99
bat-SL-CoVZXC21/2015/MG772934.1-PRC
BJ182-12/2008/EU371564.1-PRC
ZS-B/2003/AY394996.1-PRC
ZS-C/2003/AY395003.1-PRC
99
SZ16/2003/AY304488.1-PRC
SZ3/2003/AY304486.1-PRC
99
GZ02/2003/AY390556.1-PRC
CUHK-W1/Human/2003/AY278554.2-PRC
HSZ-Bb/2003/AY394985.1-PRC
2c EMC/2012/Human/JX869059.2-Saudi Arabia
2c Jordan-N3/2012/MG167/KJ614529.1-Jordan
camel/UAE B73 2015/MF598663.1-UAE
99
Hu/UAE 002 2013/Human/KY581684.1-UAE
Hu/UAE 004 2013/Human/KY581685.1-UAE
Riyadh 2 2012/Human/KF600652.1-Saudi Arabia
73
Camel/Qatar 2 2014/KJ650098.1-Qatar
MERS-CoV/2013/Human/KF192507.1-UAE
England-Qatar/2012/Human/KC667074.1-England-Qatar
England 1/2012/Human/NC 038294.1-UK
FR2019001/Pig/2019/MN056942.1-France
0.20
B
2019-nCoV
Farm / Wild animals
Human-to-human transmission
C
human-Wuhan-Hu-1/2020
25K
20K
15K
10K
5K
'forward'
'reverse'
0
5K
10K
15K
20K
25K
bat-Yunnan/RaTG13/2013
bat-SL-CoVZC45/2017
human-BJ182-12/2008
D
C
V
INDEL


S-protein
E-protein
M-protein
N-protein
PRC
TH
JPN
APR
Global
Mean binding affinity of the predicted peptides
100
80
60
40
20
0
30-44
60-74
90-104
120-134
150-164
180-194
210-224
240-254
270-284
300-314
330-344
360-374
390-404
420-434
450-464
480-494
510-524
540-554
570-584
600-614
630-644
660-674
690-704
720-734
750-764
780-794
810-824
840-854
870-884
900-914
930-944
960-974
990-1004
1020-1034
1050-1064
1080-1094
1110-1124
1140-1154
1170-1184
1200-1214
1230-1244
1-15
2-16
3-17
4-18
5-19
6-20
7-21
8-22
9-23
10-24
11-25
12-26
13-27
14-28
15-29
16-30
17-31
18-32
19-33
20-34
21-35
22-36
23-37
24-38
25-39
26-40
27-41
28-42
29-43
30-44
31-45
32-46
33-47
34-48
35-49
36-50
37-51
38-52
39-53
40-54
41-55
42-56
43-57
44-58
45-59
46-60
47-61
48-62
49-63
50-64
51-65
52-66
53-67
54-68
55-69
56-70
57-71
58-72
59-73
60-74
61-75
100
80
60
40
20
0
5-19
10-24
15-29
20-34
25-39
30-44
35-49
40-54
45-59
50-64
55-69
60-74
65-79
70-84
75-89
80-94
85-99
90-104
95-109
100-114
105-119
110-124
115-129
120-134
125-139
130-144
135-149
140-154
145-159
150-164
155-169
160-174
165-179
170-184
175-189
180-194
185-199
190-204
195-209
200-214
205-219
7-21
14-28
21-35
28-42
35-49
42-56
49-63
56-70
63-77
70-84
77-91
84-98
91-105
98-112
105-119
112-126
119-133
126-140
133-147
140-154
147-161
154-168
161-175
168-182
175-189
182-196
189-203
196-210
203-217
210-224
217-231
224-238
231-245
238-252
245-259
252-266
259-273
266-280
273-287
280-294
287-301
294-308
301-315
308-322
315-329
322-336
329-343
336-350
343-357
350-364
357-371
364-378
371-385
378-392
385-399
392-406
399-413
Sliding window of all possible 15-mer peptides