Synthetic SARS-CoV-2 (2019-nCoV), **MERS-CoV** and **SARSr-CoV** vaccine : A comparative computational analysis to propose candidate epitopes.

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Abstract

Background: Coronaviruses are normally specific to an animal taxon as host, mammals or birds depending on their species; however, these viruses can sometimes change host as a result of mutation. The HCoV-229E is one of six human coronaviruses that include HCoV-NL63, HCoV-OC43, HCoV-HKU1, MERS-CoV and SARSr-CoV (SARSr-CoV-1 and SARS-CoV-2) and is distributed globally. MERS-CoV and SARS-CoV-2 could persist on inanimate surface like (metal, glass or plastic for up to nine days) (**Huang, C.-L, 2020).** Preventive measures and drug treatment with hydrochloroquine are useful, but a good effective vaccine may be needed to stop propagation of the covid-19 pandemic. The aim of our study was to identifiy vaccine candidate epitopes by bioinformatics approaches. **Materials and Method**: The complete genomic sequences of SARS-CoV-2 were obtained from 2019 Novel Coronavirus Resource (2019nCoVR) and two databases, including the National Center for Biotechnology Information (NCBI) Global Initiative on Sharing All Influenza Data (GISAID) and TAURAU/T-bio-info-server for online bioinformatics. we will provide a brief review of viral origin, compare the sequencing data for conserved region identification, his commonest protein domain(target binding site) and consensus motif design for an potential candidate molecule (epitope) prurposition for treatment strategies for the newly identified **2019-nCoV** , SARSr-CoV strain and MERS-CoV. **Results:** Interestingly, sequence comparison between SARS-CoV-2 and another strain revealed that the residues present in the receptor-interacting motive are highly conserved with 70 % identity. we funded five important amino acids **(L455, Y473, N479, F486, Q493)** on the receptor binding domain from spike proteins responsible of contact between virus and horst **.** In the SARS-CoV_ RBD are present residues (**D480, and T487**) that allowed the interspecies infection**.** However, in SARS-CoV-2, slight modification of some

residues could improve the interaction with the human cellular receptor: **L455, F486, Q493**, and **N501.** In SARS-CoV, two main residues (479 and 487) have been associated to the recognition of the human ACE2 receptor**.** In the SARS-CoV-2, the residues corresponding to **N479** correspond to **Q493** and **T487** to **N501**. These changes in the SARS-CoV-2 represent energetically favorable changes for the interaction with the receptor. we identified the sequences of amino acids that are well conserved across many coronaviruses including 2019-nCoV and other strains, the motif **KRSFIEDLLFNKVTLADAGF** was found to be particularly well-conserved in this study and corresponds to the region around one of the known cleavage sites of the SARS virus that are believed to be required for virus activation for cell entry. This sequence motif and surrounding variations formed the basis for proposing a specific synthetic vaccine epitope this finding can make related likely rigid small molecule candidates and binding targets. **Conclusion:** This study provides information and opportunities for biological confirmation. The work can nevertheless be described in bioinformatics terms, and easily replicated by others, although new data and research on Covid-19 are emerging and evolving at an explosive rythm.

Keywords : SARS-CoV, RBD , *MERS-CoV*, épitopes, zoonose, 2019-nCoV, COVID-19.

Introduction

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is the virus responsible for the 2019 coronavirus pandemic (COVID-19). First identified in Wuhan (Hubei, China) in December 2019, it has since been declared a pandemic by the World Health Organization (WHO) in March 2020 (**Hoek, L *et al.*, 2006)**. First discovered in the 1960s, coronaviruses (Coronaviridae) are a family of enveloped positive sense single-stranded ribonucleic acid (RNA) viruses (**Kahn J *et al.*, 2005)**. Its genome ranges from 27 to 34 kilobases, which is the largest RNA viruses (**Sexton N *et al.*, 2016)**. Since 1960s, the following seven human pathogenic strains have been identified: HCoV-OC43 in Italy in 1960s (**Gerna *et al.*, 1978**), SARSr-CoV in in Guangzhou, China in 2003-2004 (**Wang *et al.*, 2005**), HCoV-NL63 in the Netherlands in 2004 **(Van Der Hoek *et al.*, 2006)**, HCoV-HKU 1 in Hong Kong (**Lau *et al.*, 2006**) and MERS-CoV the middle eastern strain in Saudi Arabia in 2004 (**Alimuddin I. Zumla and Ziad A. Memish 2014**) and SARS-CoV-1 and SARS-CoV-2 in Wuhan, China in 2019 (**Zhang *et al.*, 2020**). Each human conoravirus uses a key cell entry mechanism to infect humans or intermediate hosts (bats, Pangolin, mice, etc…) (**Ye *et al.*, 2020**).

Among the Coronaviridae family and Orthocoronavirinae subfamily, the Alphacoronavirus and Betacoronavirus are transmissible to humans. The Alpha- and Betacoronavirus strains are thought to have originated from the bat species (Rousettus les chenaultii) (**Woo *et al.*, 2007 et Lau *et al.*, 2010**). Clinical presentation can vary widely, ranging from mild cold-like symptoms to severe respiratory distress and death (**Zhou TY *et al.*, 2020**). The Alphacoronavirus strains 229E and NL63, along with the Betacoronavirus strains OC43 and HKU1, tend to cause only milder symptoms. The Betacoronavirus strains MERS-CoV (Middle East respiratory syndrome coronavirus), SARS-CoV (severe acute respiratory syndrome coronavirus), and SARS-CoV-2 are known for causing severe respiratory distress.

In recent history, several outbreaks have occurred related to the Betacoronavirus strains. Human-to-human transmission primarily occurs through close contact and through respiratory droplets (**Gerna *et al.*, 2007**). Viral transmission is admittedly increased at lower ambient temperatures. Viral-laden droplets are more effectively produced due to increased evaporation at relatively lower humidity, allowing for viral particles to remain airborne for a longer period (**Masters *et al.*, 2019**). Once viral particles enter the respiratory tract, the virus attaches to pulmonary cells followed by endocytosis and viral replication, which lead to the clinical manifestation of the disease (**Owczarek *et al.*, 2018; Zhou TY *et al.*, 2020**).

Despite the current screening and management strategies, the morbidity and mortality of COVID-19 has been astonishing worldwide except in Africa, especially in developed countries

(**Jelnov Pavel, 2020; Njenga *et al*., 2020**). Based on such observation, two perspectives are up to be explored: (i) the development of an effective vaccination for the prevention against COVID-19 infection (ii) further investigation of the underlying causes of low morbidity and mortality in the African populations. In this study, we focused on the first perspective, the vaccine development. We targeted the two (2) most virulent strains of coronaviruses (SARS-CoV, MERS-CoV) along COVID-19 involved in acute respiratory infections for a reliable comparaison. Our aim was to determine the different functional domains of these strains, especially the most conserved domain through which the virus binds to the host cell membrane receptor binding site, to identify a consensus pattern for a candidate vaccine against CoV-SARS. We hypothesized that such systematic comparison and analysis can allow to predict the interaction between the receptor-binding domain (RBD) of coronavirus spike protein and the host receptor, angiotensinconverting enzyme 2 (ACE2). Subsequently, the identification of the interaction between the key amino acids of S protein RBD and ACE2 may guide to potential therapeutic pathways.

Virus identification.

Shortly after investigations began, it was determined that a Betacoronavirus was responsible, which was identified as SARS-CoV-2 Prior to its identification, the virus was called the 2019 novel coronavirus (2019-nCoV). Some are suggesting a change of name to human coronavirus 2019 (HCoV-19) to avoid confusion with the recent strain SARSr-CoV from 2002. Here, we will refer to the new strain as SARS-CoV-2, as accepted by the WHO and the Centers for Disease Control and Prevention (CDC). (**Perlman S et al. 2020**). This newly identified human strain is thought to be related to the bat and pangolin coronavirus as well as SARS-CoV. (**Zhu N et al. 2019**). Genetic analysis has placed the virus in the genus Betacoronavirus and subgenus Sarbecovirus (lineage B), which confirms its likely origin to the bat coronavirus (BatCoV RaTG13) (**Zhou P et al. 2020**). Further analysis has revealed only one amino acid difference between SARS-CoV and the pangolin Coronavirus (Pangolin-CoV), suggesting a possible intermediate host (**Perlman S et al. 2020**).

Both SARS-CoV and MERS-CoV enter cells through an endocytosis pathway, using surface spike (S) proteins to bind to the angiotensin-converting enzyme 2 (ACE-2) and dipeptidyl peptidase 4 (DPP4) receptors on the ciliated bronchial epithelial cells and type II pneumocytes, respectively (**Masters P et al. 2019**) The molecular biology of coronaviruses. (**Cui J et al. 2019**). Once the virus enters the host cell, the viral RNA is exposed. Open reading frames 1a and 1ab (ORF1a and ORF1ab) are translated, producing polyproteins (pp1a and pp1ab). These polyproteins are later cleaved to form structural proteins for the RNA replicasentranscriptase complex, which is responsible for the replication and transcription of viral RNA. Viral

nucleocapsids are assembled and bud from the lumen of the endoplasmic reticulum Golgi intermediate compartment (ERGIC). As viral nucleocapsids encase viral RNA to produce new coronavirus virions, they are exocytosed, completing the replication cycle.

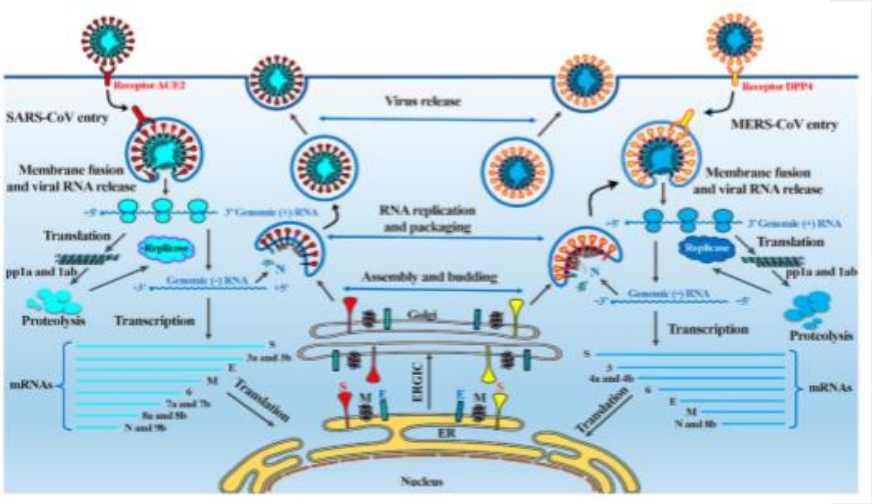

Figure 1 : Replication cycle of SARS-CoV and MERS-CoV This image details the replication cycle of SARS-CoV and MERS-CoV. Photo credit to (**Zumla et al. 2018).**

MATERIEL AND METHODS

Data Collection

The complete genomic sequences of SARS-CoV-2 were obtained from the following databases: the 2019 Novel Coronavirus Resource (2019nCoVR), the National Center for Biotechnology Information (NCBI), the Global Initiative on Sharing All Influenza Data (GISAID) and the TAURAU/T-bio-info_server for online bioinformatics. The DNA sequences of two other representative CoVs (SARS-CoVand MERS-CoV) were included for comparative analysis. The genomic information of the latest SARS-CoV-2 strains is shown is founded in **t-bio-info_server**.

RESULTS

Bioinformatic analyses

1. **Comparative Genomics**

a. Homology Analysis

The functional regions of the genome of the three strains were mapped with the NCBI homology tools. These different genes that code for proteins have roughly the same size from one strain to another. Structural particularities may justify the functional differences between the strains.

Figure 2-a : The structural representation of the different open reading frame (ORFs) of the three strains explaining their differential expressions. (adapted from the NCBI open frame reading).

In addition to the other ORFs, SARS-CoV-2 presents the ORF1 gene, which is absent in the other strains, the continuous linear arrangement of the genes in SARSrCoV and SARSCoV could eventually have an impact on the modes of action of the viruses. Importantly, the presence of the Sprike gene and at the same position in all three strains may suggest that its protein is the most common and conserved region of the β coronavirus genome. Finding an associated motif may be helpful in the search for a target therapeutic molecule.

NB: The way genes are arranged in a genome can impact the structural conformation of the proteome in an organism. This similar native conformation in both SARSrCoV and SARSCoV strains explains their recognition by the same ACE2 membrane receptor in the host. Different from the MERSCoV strain.

Strain	Orf1ab	S	ORF3a	E	M	ORF6	ORF7a	ORF8	N	ORF10
Covid-19	95%	80%	91%	100%	98%	93%	88%	94%	94%	50%
SARSr-	86%	76%	72%	94%	90%	68%	85%	40%	90%	-
MERS-CoV	50%	35%	-	36%	42%	-	-		48%	-

Figure 2b: Comparison of SARS-CoV-2 (Wuhan-Hu-1) Genome Structure with Its Closest SARSr-CoV, and MERS-CoV. Above coding sequence (CDS) region corresponding to homologous proteins between the three viruses are filled with the same coloring genome schematic to indicate homology region without homology to the predicted and up table of pairwise protein similarities (expressed as % identity) between SARS-Covid-19 and the other two viruses.

The differences and similarities of clinical characteristics between COVID-19, SARS were and MERS-CoV summarized in **Table 1.**

Table 1 : Comparison of SARS-CoV, COVID-19 and MERS-CoV

Commenté [s1]: Add references

Items	SARS	COVID-19	MERS	References
Receptor binding site	ACE2	ACE2	PPD4	Du, L., Tai, W., Zhou, Y. & Jiang, S. Vaccines for the prevention against the threat of MERS-CoV. Expert Rev. Vaccines 15, 1123–1134 (2016).
First occurrence	Nov. 16th, 2002 in Foshan, Guangdon	Dec. 07th, 2019 in Wuhan, Hubei	Saudi Arabia 2012	Du, L., Tai, W., Zhou, Y. & Jiang, S. Vaccines for the prevention against the threat of MERS-CoV. Expert Rev. Vaccines 15, 1123–1134 (2016).re
Pathogen	SARSr-CoV	SARS-CoV 2	MERS-CoV	Du, L., Yang, Y., Zhou, Y., Lu, L., Li, F. & Jiang, S. MERS-CoV spike protein: a key target for antivirals. Expert Opin. Ther. Targets 21, 131–143 (2017).
Species pathogen	β-coronavirus	β-coronavirus	β-coronavirus	Ge XY, Li JL, Yang XL, Chmura AA, Zhu G, Epstein JH, et al. Isolation and characterization of a bat SARS-like coronavirus that uses the ACE2 receptor. Nature. 2013; 503: 535-8.
DNA size (kb)	29,75	29,903	30.2	Schadt, E.E.; Turner, S.; Kasarskis, A. A window into third-generation sequencing. Hum. Mol. Genet. 2010, 19, R227–R240.
Latency	1–4 days on average	3–7 days on average	3–14 days on average	Zhao, G. et al. A safe and convenient pseudovirus-based inhibition assay to detect neutralizing antibodies and screen for viral entry inhibitors against the novel human coronavirus MERS-CoV. Virol. J. 10, 266 (2013).
Intermediate host	Paguma larvata	Pangolin, mink	dromadaire	Weiss SR, Navas-Martin S. Coronavirus pathogenesis and the emerging pathogen severe acute respiratory syndrome coronavirus. Microbiol Mol Biol Rev. 2005; 69: 635-64.
protein	14	12	11	Li, W. et al. Bats are natural reservoirs of SARS-like coronaviruses. Science 310, 676–679 (2005).
GC%	38.0	40.8	41.2	Li, W. et al. Bats are natural reservoirs of SARS-like coronaviruses. Science 310, 676–679 (2005).
Gene	13	11	10	Li, W. et al. Bats are natural reservoirs of SARS-like coronaviruses. Science 310, 676–679 (2005).
Conserved domains	Sprike	Sprike	Sprike	Wang, Q., Wong, G., Lu, G., Yan, J. & Gao, G. F. MERS-CoV spike protein: targets for vaccines and therapeutics. Antivir. Res. 133, 165–177 (2016).

2- Proteomic Comparison and Analysis

2.1. Multiple sequence alignment between SARSr-CoV, SARS-CoV-2 and MERS-CoV.

Full-length protein sequences were downloaded from the NCBI GenBank Database. This alignment shows a very high similarity: the most conserved region corresponds to the Spike protein of the S gene present in all three strains **(Fig. 3).**

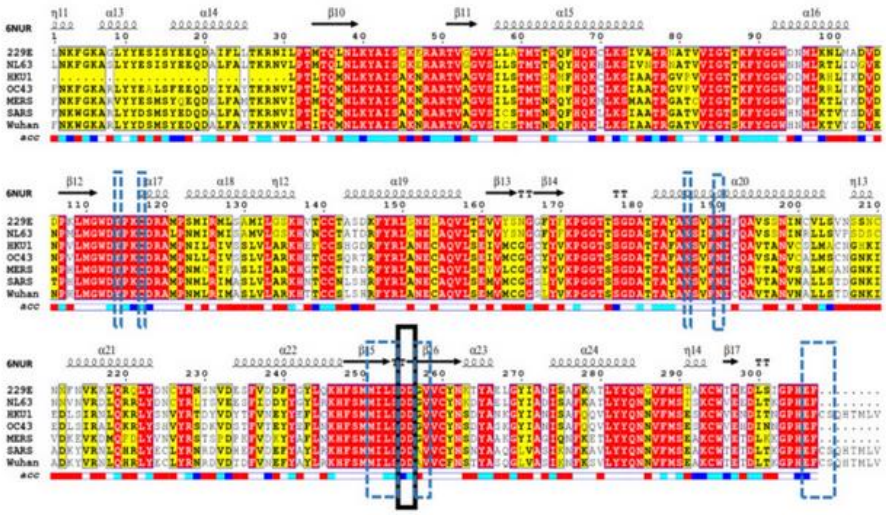

Figure 3: From the multiple alignment of sequences between the three strains, the spike region , the most conserved in all three strains corresponds to the region of interaction between the virus and the host.Spike protein analyse (RBD domain) and interaction with host receptor angiotensin-converting enzyme (ACE2 receptor), a potential coronavirus spike protein as a therapeutic target**.** SARS-CoV-2 encodes at least 27 proteins, including 15 nonstructural proteins, 4 structural proteins, and 8 auxiliary proteins (**Wu A, et al. 2020**). Spike glycoprotein (S), a structural protein located on the outer envelope of the virion, binds to the host-ACE2 receptor). The S glycoprotein of SARS-CoV, MERS-CoV, and SARSCoV-2 has 1104 to 1273 amino acids and contains an amino (N) terminal S1 subunit and a carboxyl (C)-terminal S2 subunit 13 (Fig. 4). In the S1 subunit, the receptor-binding domain (RBD), spanning about 200 residues, consists of two subdomains: the core and external subdomains. The RBD core subdomain is responsible for the formation of S trimer particles (**Yuan Y et al. 2017**). The external subdomain contains two exposed loops on the surface, which bind with ACE2 receptor (**Letko M et al. 2019**). Investigating the evolutionary relationship of the RBD sequence in spike protein will help understand the virus origin trends and therapeutic pathway.

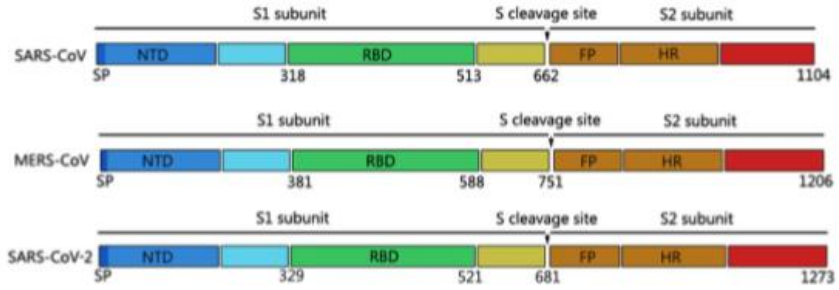

Fig. 4: Structural diagrams of spike glycoproteins of **SARSr-CoV, MERS-CoV,** and **SARS-CoV-2.** All spike proteins of coronaviruses contain **S1** subunit and **S2** subunit, which were divided by the **S** cleavage sites. **FP**, fusion peptide; **HR**, heptad repeat 1 and heptad repeat 2; **RBD**, receptor-binding domain.

2-1 Structural analyse of Spike-ACE2 (RBD-ACE2) complexes

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MN938384SARSCoV2 318RVQPTESIVRFPNITNLCPFGEVFNATRFASVYAWNRKRISNCVADYSVLYNSASFSTFKCYGVSPTKLNDL 390
MN996532batCoV   318-----d----------t----------t----------- 390
MG772933batCoV   318-----q-v--------v---hk-------p-----e-tk--d-i---t-f---t----------s--i-- 390
NC004718SARS     318--v-sgdv----------k-p-----e--k----------tf----------a------ 390

MN938384SARSCoV2 391CFTNVYADSFVIRGDEVRQIAPGQTGKIADYNYKLPDDFTGCVIAWNSNNLDSKVGGNYNYLYRLFRKSNLK 463
MN996532batCoV   391----------t----------khi-a-e---f---------a--- 463
MG772933batCoV   391--s----t-l--fs----v------v----------takq-vg.....--f--sh-stk-- 463
NC004718SARS     391-s--------vk--d----------v----------m---l---tr-i-atst-----k--yl-hgk-r 463

MN938384SARSCoV2 464PFERDISTEIYQAGSTPCNGVEGFNCYFPLQSYGFQPTNGVGYQPYRVVVLSFELLHAPATVCGPKK    531
MN996532batCoV   464----------k----qt-l---y--yr---y--d---h----------n----------    531
MG772933batCoV   464-----l-sde.....---r......t-st-d-n-nvple--at----------n---------l    512
NC004718SARS     484-------nvpfspdgk--tppa.l---w--nd---yt-t-i----------n---------l    530
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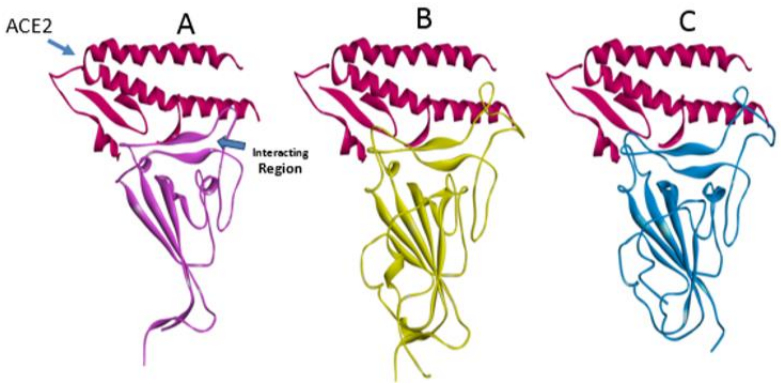
Figure 5: Receptor Binding Domain of the spike protein sequence alignment of SARS-CoV-2 and other related coronaviruses.

Sequence alignment for the interacting domain of SARS-CoV-2 (MN938384), Bat/MERS-CoV (MN996532 and MG772933) and SARS-CoV (NC004718). The key amino acids described for the interaction with ACE2 receptor (in red) and others amino acid related with the interaction in SARS-CoV2 (in blue) are shown. (Lines (-) same amino acid, dots (.) deletion) aligned buy I-TASSER server. Motif scanHomology analysis of the spike proteins of SARS-CoV2, and another strain are shown **(Fig. 5).**

Interestingly, sequence comparison between SARS-CoV-2 and another strain revealed that the residues present in the receptor-interacting motive are highly conserved with 70% identity, with nine shared residues. In the SARS-CoV receptor binding domain (RBD) have residues that allow the interspecies infection, known as**, D480, and T487 (Lu et al., 2015).** However, in SARS-CoV-2, slight modification of some residues could improve the interaction with the human cellular receptor: **L455, F486, Q493**, and **N501**. In SARS-CoV, two main residues (479 and 487) have been associated with the recognition of the human ACE2 receptor **(Lu et al., 2015).** These residues suffered a punctual mutation from civet to human, **K479N** and **S487T (Li, 2013).** In the SARS-CoV-2, the residues corresponding to N479 correspond to Q493 and T487 to N501. These changes in the SARS-CoV-2 represent energetically favorable changes for the interaction with the receptor. The local environment present in the ACE2 receptor allows these mutations to produce a significant number of electrostatic stabilizing interactions**.** Furthermore, as mentioned previously, the presence of the two capping loops in the binding domain is likely producing a stabilization effect over the interaction with the cellular receptor. Our models showed that these capping loops appear in both human-infecting viruses but are absent in the bat virus. The data showed here strongly suggested that these capping loops had an increased electrostatic interactions between the spike protein and the cellular receptor. In SARS-CoV, the residues present in these capping loops showing direct interaction with the receptor are **R426, S432, T433, Y436, P462, D463, S472, and N473 and in SARS-CoV-2 are V445, Y449, Y473, Q474, A475, E484, G485, F486, and N487**. Thus, these loops could play an important role together with the punctual mutations being an interesting clue to determine the host receptor specificity for the viral spike protein.



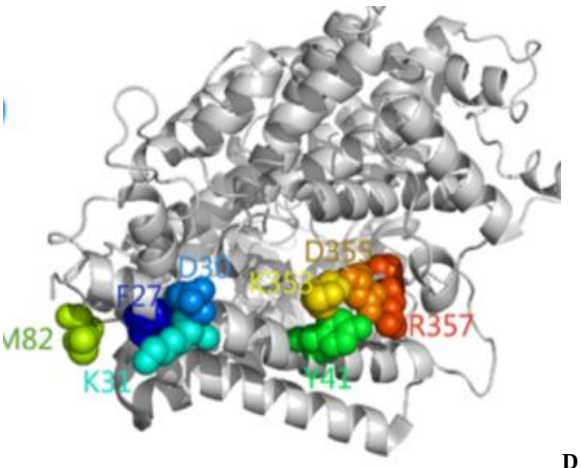


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Figure 6 : Coronavirus spike proteins 3D proteins structures with a keys amino acids.

The spike proteins in complex with the RBD of ACE2 (dark/pink) are shown A) SARSr-CoV, B) MERS-CoV, and D) Human ACE2 critical for the binding with SARS-CoV-2 spike RBD is shown. The key residues located in the interface of ACE2 possibly in combination with spike RBD are shown as a sphere and colored . Consistent with SARS-CoV binding to human ACE2, the residues 31, 41, 82, 353, 355, and 357 on the receptor locate in the interface when interacting with SARS-CoV-2 spike protein **Met82** in human receptor may interact with position 472 of RBD. Residues **K479** and **S487** in civet SARS-CoV can effectively recognize human ACE2, but bind with human ACE2 much less efficiently. The residue **Thr487** in RBD binds to **Tyr41** and **Lys353** in human **ACE2** from this perspective of **Asn501** in RBD domain with the sites 41 and 353 of ACE2 receptor. (**Li W et al. 2005).** Molecular markers for the identification of SARS-CoV-2 and SARS-CoV, and also help to develop new drugs against SARS-CoV-2.

2.3- Searching for conserved patterns (epitopes) on spike domain for therapeutic targeting :

The multiple alignment of the sequences of these domains of its different strains performed with Blastp, MAFFT , CLUSTALW and scan pattern shows YP_009724390.1, YP_009047204.1, AAR07628.1, AIA62339.1, ACJ60703.1, AAV98001.1

ADE34823.1, IDACB69883.1, SARS-COVr spike glycoprotein, ATO98218. 1. That the **K**RS**F**IE**D**LL**F**NK**V**TLA**DA**G**F** pattern has been shown to be particularly well conserved on theRBD domain of the spike gene and corresponds to the region

around one of the known cleavage sites of the SARS virus which would be necessary for activation of the virus for cell entry. This sequence motif and the surrounding variations can serve as a basis for proposing a specific synthetic vaccine epitope. Against covid-19 and all SARS

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FGGFNFSQILPDPSKPSKRSFIEDLLFNKVTLADAGFIKQYGDC  - 2019-nCoV CoV spike glycoprotein
FGGFNFSQILPDPLKPTKRSFIEDLLFNKVTLADAGFMKQYGEC  - [Bat SARS-like CoV] ID:ATO98218.1
FGGFNFSQILPDPLKPTKRSFIEDLLFNKVTLADAGFMKQYGEC  - [SARS CoV BJ302] ID: AAR07628.1
FGGFNFSQILPDPSKPSKRSFIEDLLFNKVTLADAGFIKQYGDC  - [BtRf-BetaCoV/HeN2013] ID:AIA62339.1
YSGFNFSQILPDPLKPTKRSFIEDLLFNKVTLADAGFMKQYGEC  - SARS-CoV spike glycoprotein
YSGFNFSQILPDPLKPTKRSFIEDLLFNKVTLADAGFMKQYGEC  - [recombinant  CoV] ID:ACJ60703.1
YSGFNFSQILPDPLKPTKRSFIEDLLFNKVTLADAGFMKQYGEC  - [SARS CoV BJ182-] IDACB69883.1
FGGFNFSQILPDPLKPTKRSFIEDLLFNKVTLADAGFMKQYGEC  - [SARS CoV C028]ID:AAV98001.1
FGGFNFSQILPDPSKPTKRSFIEDLLFNKVTLADAGFMKQYGDC  - [Bat SARS HKU3-13] ID:ADE34823.1
FGGFNFSQILPDPLKPTKRSFIEDLLYNKVTLADAGFMKQYADC  - [BtRf-BetaCoV/HeN2013]ID:AIA62339.1
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Fig7 : multiple alignment des sequence du domaine RDB du gene spike entres les differentes souches de SARS. Montrant un epitope consencus entre les souches alignées

DISCUSSION

As a large number of people have left Wuhan, the control of the epidemic situation is extremely urgent, and the treatments of COVID-19 are imminent. On Feb. 14th, 2020, there were more than 54,000 confirmed patients in Hubei province, China . Due to the lack of effective antiviral drugs, the prognosis of patients solely depends on their age and physical condition (Chen, Y et al. 2020). Although it was reported that the clinically recovered patients exceed the number of dead, the majority of the patients are still not cured in hospital.

During our study,In the SARS-CoV_ RBD are present residues (**D480, and T487**) that allowed the interspecies infection**.** However, in SARS-CoV-2, slight modification of some residues could improve the interaction with the human cellular receptor (**L455, F486, Q493**, and **N501).** In SARS-CoV, two main residues (**N479** and **T487**) have been associated to the recognition of the human ACE2 receptor**.** These changes in the SARS-CoV-2 represent energetically favorable changes for the interaction with the receptor. we identified the sequences of amino acids that are well conserved across many coronaviruses including 2019-nCoV and over strains the motif **K**RS**F**IE**D**LL**F**NK**V**TLA**DA**G**F** was found to be particularly well conserved and corresponds to the region around one of the known cleavage sites of the SARS virus that are believed to be required for virus activation for cell entry. According to **(Wan, Y et al. 2020),** the potential adaptive mutation of SARS-CoV-2 makes it difficult for vaccine development. Therefore, it is urgent for us to develop more sensitive inspection methods and effective drugs. Seven type of CoVs have been identified to cause human disease**.** In addition

the potential adaptive mutation of SARS-CoV-2 makes it difficult for vaccine development. Therefore, it is urgent for us to develop more sensitive inspection methods and effective drugs. Seven type of CoVs have been identified to cause human disease. The tree highly pathogenic viruses, SARS-CoV and MERS-CoV and SARS-CoV-2 cause severe respiratory syndrome in humans. The other four human CoVs (HCoV-NL63, HCoV-229E, HCoV-OC43 and HKU1) induce only mild upper respiratory diseases, although some of them can cause severe infections in infants, young children, and elderly individuals (**Su, S et al. 2016**). The latest SARS-CoV-2. It has been reported that SARS-CoV-2 shared almost 80% of the genome with SARS-CoV (**Fields, B. N et al. 2006**). Our results also showed that almost all encoded proteins of SARS-CoV-2 are homologous to SARSr-CoV and MERS-CoV proteins (**Fig4)**. Hence, clinical drugs and therapies for treating SARS may be used as a reference for COVID-19 treatment . In addition to the well-known SARS-CoV, MERS-CoV, as one Merbecovirus subgenus of β-CoVs, is also extremely invasive. MERS-CoV is the pathogen of the Middle East Respiratory Syndrome, which can infect both humans and animals, and can be transmitted through camels (**Chu, D.K.W et al. 2014**). It mainly occurs in Saudi Arabia and has a high mortality rate (**De Wit et al. 2016**). Studies had demonstrated that the clinical course of SARS and MERS was highly similar, and SARS and MERS may have similar pathogenesis. The genome sequence of SARS-CoV-2 also shows some similarities to that of MERS-CoV. It will be very interesting to study the relationship among SARS-CoV, MERS-CoV, and SARS-CoV-2 that may be exploited for future developing broad-spectrum antiviral therapies. Although more and more studies for SARS-CoV-2 have sprung up since the outbreak of this epidemic COVID-19. The proteins sequence aligned shown. The spike (S) protein and N protein confer stability to the viral particle . The N protein is a structural protein involved in virion a ssembly,and plays apivotal role in virus transcription and assembly efficiency(**Perlman, S et al. 2009**). S protein can bind to the cellular receptors of sensitive cells and mediate infection of their target cells, after which it begins to replicate in the cytoplasm . CoV can enter host cells through the interaction between CoV S protein and its host receptor angiotensin-converting enzyme 2 (ACE2), which is isolated from SARS-CoV–permissive Vero-E6 cells. The receptor-binding motif (RBM) of S protein can directly contact ACE2 (**Zhou, P. et al. 2020**). AEC2 have been identified to be key binding residues and functional receptor for SARS-CoV, and it can also protect alveolar cells . The binding of spike protein to ACE2 and the subsequent down regulation of this receptor contribute to severe alveolar injury during SARS (**Zhou, P et al. 2020**). The down regulation of ACE2 results in the excessive production of angiotensin II by the related enzyme ACE, and the stimulation of type1AangiotensinII receptor(AGTR1A) can lead to the increase of pulmonary vascular permeability, which potentially explains the increased lung pathology when the expression of ACE2 is decreased. The virus was shown to replicate effectively in ACE2-transfected, but not in mock-transfected T cells. Antibodies targeting ACE2 can block viral

replication in Vero-E6 cells (**Zhu N et al. 2019**) Recently, Ji et al. demonstrated that the receptor binding domain of SARS-CoV-2 was capable of binding ACE2 in the context of the SARS-CoV spike protein **Perlman, S et al. 2009**. Among SRAS-CoV spike protein's fourteen residues predicted to interact directly with human ACE2 as the receptor for SARS-CoV, eight amino acids are well conserved in homology SARS-CoV-2 spike protein. At the same time, Wan et al. showed that SARS-CoV-2 uses ACE2 receptors to infect humans, bats, civets, monkeys, and swine, but not mice (**Wan, Y.et al. 2020**). Compared to previously reported SARS-CoV strains, SARS-CoV-2 uses ACE2 receptors more efficiently than human SARS-CoV (year 2003), but less efficiently than human SARS-CoV (year 2002) . The mutation of proteins determines two important characteristics of the SARS-CoV-2: A higher ability to infect and enhanced pathogenicity than the bat-like SARS-CoV, but a lower pathogenicity than SARS-CoV (**Huang, C. et al. 2020**). In-depth understanding the underlying pathogenic mechanisms of SARS-CoV-2 will reveal more targets for better therapy of COVID-19. based on our comparison, we propose some key questions to be clarified in futures studies like recommandation below.

Recommandations :

I recommend to search to answered the following questions listed below :

1- What is the effect of the surface epitope and receptor binding domain of S protein of SARS-CoV-2 on the virus'infectivity?
2- Does SARS-CoV-2 ***orf8*** and ***orf10*** proteins, which have no homology proteins in SARS-CoV, play roles in the infectivity and pathogenicity of SARS-CoV-2?
3- Can the susceptibility of asymptomatic carriers be judged by detecting the serum reactivity level of N protein?
4- What is the percentage of COVID-19 patients have been infected with SARS and produced antibodies?
5- Does traditional Chinese medicine have any effect on the treatment of COVID-19 caused by SARS-CoV-2?

REFERENCES

1- **Al-Tawfiq, J.A.; Zumla, A.; Memish, Z.A**. Travel implications of emerging coronaviruses: SARS and MERS-CoV. Travel. Med. Infect. Dis. 2014, 12, 422–428. [CrossRef]

2- **Alimuddin I. Zumla and Ziad A.** Memish. Middle East respiratory syndrome coronavirus: epidemic potential or a storm in a teacup? Eur Respir J 2014; 43: 1243–1248 | DOI: 10.1183/09031936.00227213

3- **Chen, Y.; Liu, Q.; Guo, D**. Emerging coronaviruses: Genome structure, replication, and pathogenesis. J. Med. Virol. 2020, 92, 418–423

4- **Chu, D.K.W.; Poon, L**.; Gomaa, M.; Shehata, M.; Perera, R.A.; Abu Zeid, D.; El Rifay, A.S.; Coronavirus disease (COVID-19) pandemic. (2020). Accessed: March 20, 2020: https://www.who.int/emergencies/diseases/novel-coronavirus-2019.

5- **Coronavirus disease** 2019 (COVID-19): situation summary. (2020). Accessed: March 20, 2020: https://www.cdc.gov/coronavirus/2019-ncov/cases-updates/summary.html.

6- **Cui Li F, Shi Z**: Origin and evolution of pathogenic coronaviruses. Nat Rev Microbiol. 2019, 17:181-192. 10.1038/s41579-018-0118-9

7- **De Wit, E.; van Doremalen, N.; Falzarano, D.; Munster, V.J. SARS and MERS:** Recent insights into emerging coronaviruses. Nat. Rev. Microbiol. 2016, 14, 523

8- **Early Transmission Dynamics in Wuhan, China, of Novel Coronavirus-Infected Pneumonia** . (2020). Accessed: February 1, 2020:

9- https://www.nejm.org/doi/full/10.1056/NEJMoa2001316.

10- **Esper, F., C. Weibel, D. Ferguson, M. L. Landry, and J. S. Kahn. 2006.** Coronavirus HKU1 infection in the United States. Emerg. Infect. Dis. 12:775–779. [PMC free article] [PubMed] Evidence of recombination in coronaviruses implicating pangolin origins of nCoV-2019 . (2020). Accessed: February 14, 2020:

11- https://www.biorxiv.org/content/10.1101/2020.02.07.939207v1.

12- **Fields, B. N., D. M. Knipe, and P. M. Howley (ed.). 1996. Fields virology, 3rd ed**. Lippincott-Raven, Philadelphia, PA.

13- **Fei Zhou TY**, Ronghui Du, Guohui Fan, Ying Liu, Zhibo Liu, Jie Xiang, Yeming Wang, Bin Song, Xiaoying Gu, Lulu Guan, Yuan Wei, Hui Li, Xudong Wu, Jiuyang Xu, Shengjin Tu, Yi Zhang, Hua Chen, Bin Cao,. Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: a retrospective cohort study. The Lancet. 2020 March 9, 2020.

14- **Gerna G, Cattaneo E,** Cereda P, Revello MG. Seroepidemiologic study of human coronavirus OC43 infections in Italy. Boll Ist Sieroter Milan. 1978;57(4):535-542.

15- **Gerna, G., E. Percivalle, A. Sarasini, G. Campanini, A. Piralla, F. Rovida, E. Genini, A.** Marchi, and F. Baldanti. 2007. Human respiratory coronavirus HKU1 versus other coronavirus infections in Italian hospitalised patients. J. Clin. Virol. 38:244–250. [PubMed]

16- **Hoek, L. van der, P. Krzysztof, and B. Berkhout. 2006**. Human coronavirus NL63, a new respiratory virus. FEMS Microbiol. Rev. 30:760–773. [PubMed]

17- **Huang, C.; Wang, Y.; Li, X.; Ren, L.; Zhao, J.; Hu, Y.; Zhang, L.; Fan, G.; Xu, J.; Gu, X.; et al**. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. Lancet 2020, 395, 497–506

18- **Jelnov, Pavel, 2020.** "Confronting COVID-19 Myths: Morbidity and Mortality," GLO Discussion Paper Series 516, Global Labor Organization (GLO). <https://ideas.repec.org/p/zbw/glodps/516.html>

19- **Kahn J, McIntosh K: History and recent advances in coronavirus discovery. Pediatr Infect Dis** J. 2005, 24:S223-S227. 10.1097/01.inf.0000188166.17324.60

20- **Kaye, H. S., H. B. Marsh, and W. R. Dowdle. 1971**. Seroepidemiologic survey of coronavirus (strain OC 43) related infections in a children's population. Am. J. Epidemiol. 94:43–49. [PubMed]

21- **Lau S, Poon R, Wong B, et al.**: Coexistence of different genotypes in the same bat and serological characterization of Rousettus bat coronavirus HKU9 belonging to a novel Betacoronavirus subgroup. J Virol. 2010, 84:11385-11394

22- **Letko M, Munster V**. Functional assessment of cell entry and receptor usage for lineage B βcoronaviruses, including 2019-nCoV. bioRxiv. 2020.

23- https://doi.org/10.1101/2020.01.22.915660

24- **Li W, Zhang C, Sui J, et al**. Receptor and viral determinants of SARScoronavirus adaptation to human ACE2. EMBO J. 2005;24(8): 1634-1643

25- **Lia Van Der Hoek,** Krzysztof Pyrc, Ben Berkhout. Human coronavirus NL63, a new respiratory virus. FEMS Microbiology Reviews. 2006;30(5): 760-773.

26- Lau, S. K., Woo, P. C., Yip, C. C., Tse, H., Tsoi, H. W., Cheng, V. C., Lee, P., Tang, B. S., Cheung, C. H., Lee, R. A., So, L. Y., Lau, Y. L., Chan, K. H., & Yuen, K. Y. (2006). Coronavirus HKU1 and other coronavirus infections in Hong Kong. Journal of clinical microbiology, 44(6), 2063–2071. https://doi.org/10.1128/JCM.02614-05

27- Lu G, Wang Q, Gao GF. Bat-to-human: spike features determining 'host jump' of coronaviruses SARS-CoV, MERS-CoV, and beyond. Trends Microbiol. 2015;23: 468-78.

28- Masters P: The molecular biology of coronaviruses. Adv Virus Res. 2006, 66:193-292. 10.1016/S0065-3527(06)66005-3 11.

29- M. Kariuki Njenga, Jeanette Dawa, Mark Nanyingi, John Gachohi, Isaac Ngere, Michael Letko, C. F. Otieno, Bronwyn M. Gunn, and Eric Osoro. Why is There Low Morbidity and Mortality of COVID-19 in Africa? Am. J. Trop. Med. Hyg., 00(0), 2020, pp. 1–6. doi:10.4269/ajtmh.20-0474

30- Perlman S: Another decade, another coronavirus. N Engl J Med. 2020, 382:760-762. 10.1056/NEJMe2001126

31- Perlman,S.;Netland,J.Coronavirusespost-SARS:Updateonreplicationandpathogenesis.Nat. Rev. Microbiol. 2009,7,439–450

32- Sexton N, Smith E, Blanc H, Vignuzzi M, Peersen O, Denison M: Homology-Based identification of a mutation in the coronavirus RNA-dependent RNA polymerase that confers resistance to multiple mutagens. J Virol. 2016, 90:7415-7428.

33- Siu, L.Y.; Guan, Y.; Webby, R.J.; et al. MERS coronaviruses in dromedary camels, Egypt. Emerg. Infect. Dis. 2014, 20, 1049.

34- Tao Zhang, Qunfu Wu and Zhigang Zhang. Probable Pangolin Origin of SARS-CoV-2 Associated with the COVID-19 Outbreak. Current Biology 2020,30: 1346–1351

35- Owczarek, K., Szczepanski, A., Milewska, A. et al. Early events during human coronavirus OC43 entry to the cell. Sci Rep 8, 7124 (2018). https://doi.org/10.1038/s41598-018-25640-0

36- **Su, S.; Wong, G.; Shi, W.; Liu, J.; Lai, A.C.; Zhou, J.; Liu, W.; Bi, Y.; Gao, G.F**. Epidemiology, genetic recombination, and pathogenesis of coronaviruses. Trends. Microbiol. 2016, 24, 490–502

37- **Wan, Y.; Shang, J.; Graham, R.; Baric, R.S.; Li, F**. Receptor recognition by novel coronavirus from Wuhan: An analysis based on decade-long structural studies of SARS. J. Virol. 2020.

38- **Wan, Y.; Shang, J.; Graham, R.; Baric, R.S.; Li, F**. Receptor recognition by novel coronavirus from Wuhan: An analysis based on decade-long structural studies of SARS. J. Virol. 2020

39- **Wang M, Lau S, et al.:** Comparative analysis of twelve genomes of three novel group 2c and group 2d coronaviruses reveals unique group and subgroup features. J Virol. 2007, 81:1574-1585

40- **Wang, M., Yan, M.,** Xu, H., Liang, W., Kan, B., Zheng, B., Chen, H., Zheng, H., Xu, Y., Zhang, E., Wang, H., Ye, J., Li, G., Li, M., Cui, Z., Liu, Y. F., Guo, R. T., Liu, X. N., Zhan, L. H., Zhou, D. H., … Xu, J. (2005). SARS-CoV infection in a restaurant from palm civet. Emerging infectious diseases, 11(12), 1860–1865. https://doi.org/10.3201/eid1112.041293

41- **Wu A, Peng Y, Huang B, et al**. Genome composition and divergence of the novel coronavirus (2019-nCoV) originating in China. Cell Host Microbe. 2020. https://doi.org/10.1016/j.chom.2020. 02.001

42- **Yuan Y, Cao D, Zhang Y, et al**. Cryo-EM structures of MERS-CoV and SARS-CoV spike glycoproteins reveal the dynamic receptor binding domains. Nat Commun. 2017;8:15092.

43- **Zhou P, Yang Z, Wang X, et al.:** Discovery of a novel coronavirus associated with the recent pneumonia outbreak in humans and its potential bat origin. Nature. 2020, 579:270-273. 10.1038/s41586-020-2012-7

44- **Zhu N, Zhang D, Wang W, et al.**: A novel coronavirus from patients with pneumonia in China, 2019. N Engl J Med. 2020, 382:727-733. 10.1056/NEJMoa2001017

45- **Zumla A, Hui D, Perlman S**: Middle East respiratory syndrome. Lancet. 2015, 386:995-1007. 10.1016/S0140-6736(15)60454-8

49. **Ye, Z. W.,** Yuan, S., Yuen, K. S., Fung, S. Y., Chan, C. P., & Jin, D. Y. (2020). Zoonotic origins of human coronaviruses. International journal of biological sciences, 16(10), 1686–1697. https://doi.org/10.7150/ijbs.45472