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Insights into SARS-CoV-2 genome, structure, evolution, pathogenesis and therapies: Structural genomics approach

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ABSTRACT

The sudden emergence of severe respiratory disease, caused by a novel severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has recently become a public health emergency. Genome sequence analysis of SARS-CoV-2 revealed its close resemblance to the earlier reported SARS-CoV and Middle East respiratory syndrome coronavirus (MERS-CoV). However, initial testing of the drugs used against SARS-CoV and MERS-CoV has been ineffective in controlling SARS-CoV-2. The present study highlights the genomic, proteomic, pathogenesis, and therapeutic strategies in SARS-CoV-2 infection. We have carried out sequence analysis of potential drug target proteins in SARS-CoV-2 and, compared them with SARS-CoV and MERS viruses. Analysis of mutations in the coding and non-coding regions, genetic diversity, and pathogenicity of SARS-CoV-2 has also been done. A detailed structural analysis of drug target proteins has been performed to gain insights into the mechanism of pathogenesis, structure-function relationships, and the development of structure-guided therapeutic approaches. The cytokine profiling and inflammatory signalling are different in the case of SARS-CoV-2 infection. We also highlighted possible therapies and their mechanism of action followed by clinical manifestation. Our analysis suggests a minimal variation in the genome sequence of SARS-CoV-2, may be responsible for a drastic change in the structures of target proteins, which makes available drugs ineffective.

1. Introduction

Coronavirus disease (COVID-19) is an infectious disease caused by a novel severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) that initially started in Wuhan province in China and has now affected > 200 countries worldwide and declared a Pandemic [1,2]. The virus primarily affects the respiratory system causing flu-like illness with symptoms such as a cough, fever, and in more severe cases, difficulty breathing [3]. As per the statistics available, mortality is high in older age group individuals (> 60 years of age) and people with other morbid conditions. In addition to acute respiratory distress syndrome and respiratory failure, COVID-19 is now known to manifest as systemic inflammation, leading to sepsis, acute cardiac injury, and heart failure and multi-organ dysfunction in patients at high risk [4].

COVID-19 has been declared a pandemic that has currently affected > 200 countries across the world. As on the 14th of June 2020,

Abbreviations: SARS-CoV, Severe acute respiratory syndrome coronavirus; MERS-CoV, Middle-east respiratory syndrome coronavirus; COVID-19, Coronavirus disease 2019; IL, Interleukin; TNF, Tumor necrosis factor; IFNγ, Interferon; G-CSF, Granulocyte-colony stimulating factor; S, Spike protein; M, Membrane protein; N, Nucleocapsid protein; E, Envelope protein; Nsp, Nonstructural protein; ORF, Open reading frame; UTR, Untranslated region; RBD, Receptor binding domain; ACE2, Angiotensin-converting enzyme 2; TMPRSS2, transmembrane protease serine 2; WHO, World Health Organization; FDA, Food and Drug Administration; JAK, Janus Kinase; STAT, Signal transducer and activator of transcription protein

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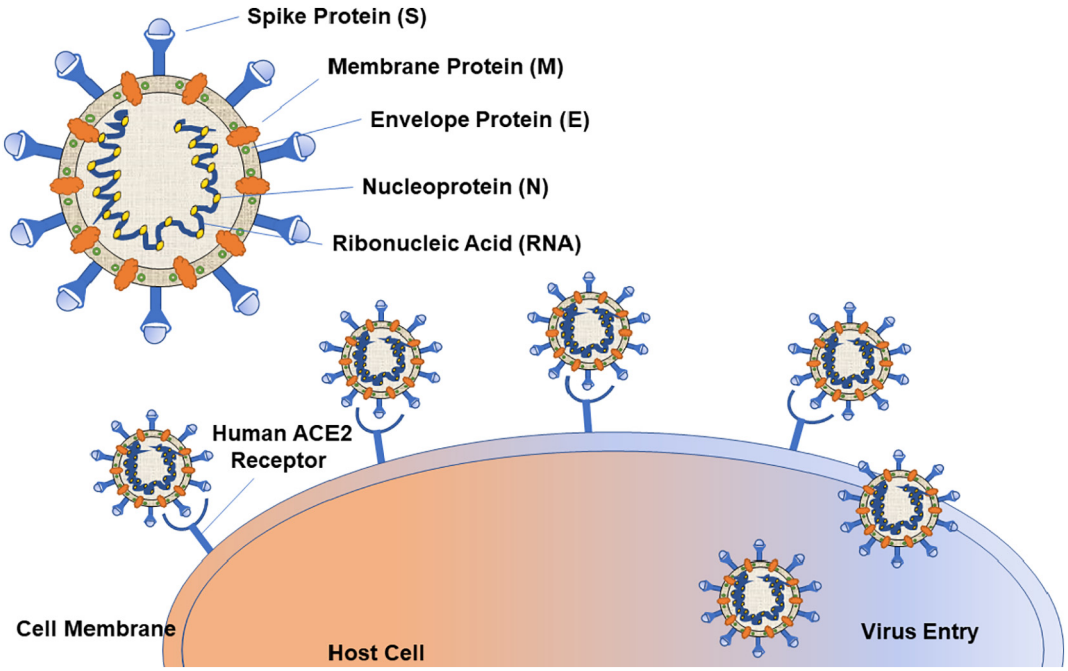

Fig. 1. Schematic representation of the SARS-CoV-2 structure and its mode of host entry.

about 8 million people have been infected so far (https://www.worldometers.info/coronavirus/). There have been more than 0.44 million deaths so far, at an average rate of 5000 deaths each day in the last three months. While more than 4 million of people have been recovered completely, 3.4 million continues to be active cases and more than 54,000 people in critical condition, requiring respiratory assistance. The countries that have been affected most are USA, Brazil, Russia, Spain, UK, India, and Italy. While the USA has the greatest number of cases with over 2.1 million being infected and has mortality with over 110700 deaths so far. China, the country where it all began in December, is now on the downslope reporting only single-digit cases and no deaths being reported. The majority of the countries around the world were in lockdown to halt human to human transmission of the virus to reduce the spread of infection. As the number of COVID-19 cases is increasing, the World Health Organization has rightly declared it as a global health emergency and pandemic [5].

Coronaviruses (CoVs) are a group of enveloped viruses, having a positive single-stranded RNA genome and pathogenic [6]. COVID-19 is caused by the SARS-CoV-2 is a more pathogenic form in comparison to previously identified SARS-CoV (2002) and Middle East respiratory syndrome coronavirus (MERS-CoV, 2013). There is an urgent need to study the virus more holistically to understand the mechanism of pathogenesis, its virulence, and to develop effective therapeutic strategies [7].

CoVs belong to the Coronaviridae family of order Nidovirales. They have been classified into four genera that include α-, β-, γ-, and δ-coronaviruses [8]. Among them, α- and β- CoVs infect mammals, γ-coronaviruses infect avian species, and δ-coronaviruses infect both mammals and aves. SARS-CoV, mouse hepatitis coronavirus (MHV), MERS-CoV, bovine coronavirus (BCoV), bat coronavirus HKU4, and human coronavirus OC43, including SARS-CoV-2, are belonging to β-coronaviruses [9]. All three CoVs, SARS-, MERS-, and SARS-CoV-2 are transmitted through zoonotic transmission and spread among humans through close contact. The primary reproduction number (R0) of the person-to-person spread of SARS-CoV-2 is about 2.6, which means that the infected cases grow at an exponential rate [10].

CoVs have the largest RNA viral genome, ranging from 26 to 32 kb in length [11]. The SARS-CoV-2 genome share about 82% sequence identity with SARS-CoV and MERS-CoV and > 90% sequence identity for essential enzymes and structural proteins. This high level of the sequence revealed a common pathogenesis mechanism, thus, therapeutic targeting. Structurally, SARS-CoV-2 contains four structural proteins, that include spike (**S**), envelope (**E**), membrane (**M**), and nucleocapsid (**N**) proteins. These proteins share high sequence similarity to the sequence of the corresponding protein of SARS-CoV, and MERS-CoV.

CoVs rely on their spike (S) proteins for binding to the host cell-surface receptor during host cell entry [12]. The S protein binds to the host receptor through the receptor-binding domain (RBD) in the S1 subunit, followed by the fusion of the S2 subunit to the cell membrane. Different cell surface receptors recognize RBD of S proteins of SARS-CoV and MERS-CoV. MERS-CoV recognizes the dipeptidyl peptidase 4 receptor. Whereas, SARS-CoV and SARS-CoV-2 recognize the ACE2 receptor to bind with the viral S protein [13]. These CoVs mainly differ in their mechanism of host entry, suggesting possible changes in the residual composition of S protein that may dictate host entry.

Design and development of SARS-CoV-2 specific direct-acting antiviral drugs can be made possible by targeting conserved enzymes such as main protease or 3C-like protease (Mpro or 3CLpro), papain-like protease (PLpro), non-structural protein 12 (nsp12) and RNA-dependent RNA polymerase (RdRP) [14]. A comparative genomics-based approach can be used to understand the molecular basis of pathogenesis with possible implications in the development of a therapeutic strategy against COVID-19.

Out of the seven pathogenic CoVs, many of them including, HCoV-NL63, HCoV-229E, HCoV-OC43, and HCoV-HKU1 cause mild clinical symptoms. SARS-CoV, MERS-CoV, and the newly identified SARS-CoV-2 infection causes severe respiratory illness and resulting in death in comorbid individuals. Potential adaptive mutations in the SARS-CoV-2 genome possibly made it highly pathogenic and difficult for drug and vaccine development [15]. Here, we provide comprehensive insights into the genome, proteome, structural features of potential drug targets, and molecular mechanisms of pathogenesis of these viruses in a comparative manner. We further highlighted the cytokine profiles, inflammatory pathways, followed by the underlying mechanism of action of current therapeutics.

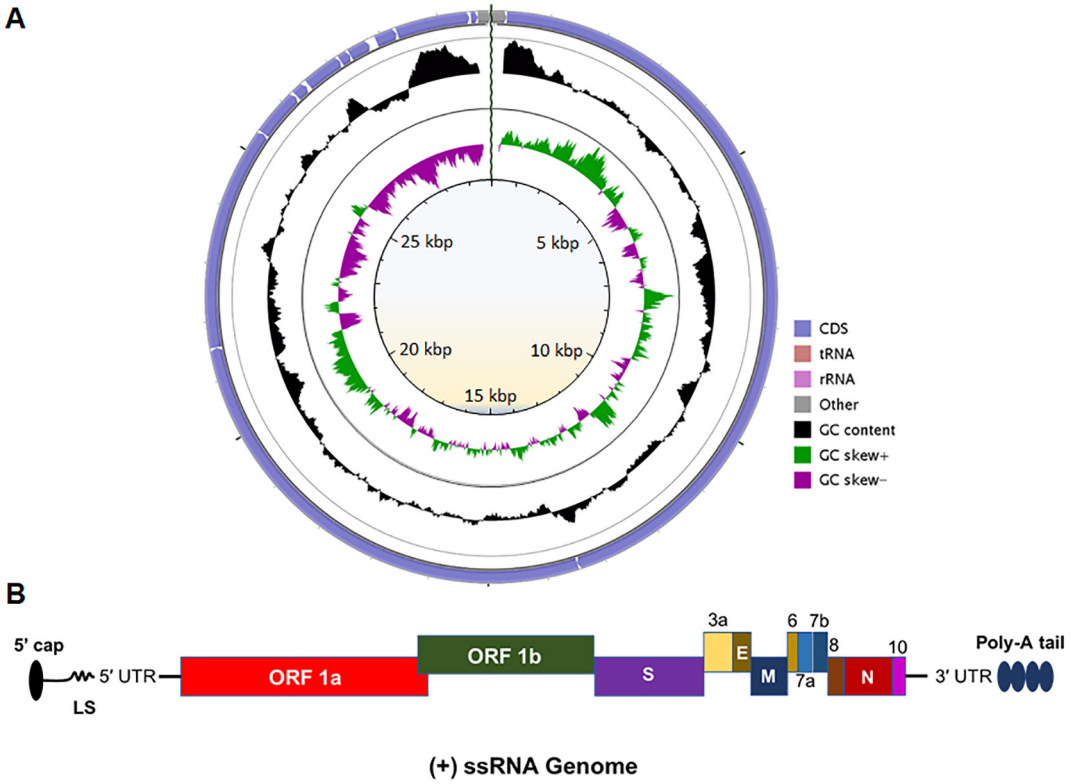

Fig. 2. Genome architecture of SARS-CoV-2. A. Representation of the reference genome of SARS-CoV-2 showing the protein-coding regions and GC content of the genome. **B.** Representation of 5′ capped mRNA has a leader sequence (LS), poly-A tail at 3′ end, and 5′ and 3′ UTR. It consists of ORF1a, ORF1b, Spike (S), ORF3a, Envelope (E), Membrane (M), ORF6, ORF7a, ORF7b, ORF8, Nucleocapsid (N), and ORF10 (Ref 31).

2. Molecular basis of pathogenesis

Like SARS-CoV and MERS-CoV, SARS-CoV-2 is also known to infect humans and cause severe respiratory disease. Whereas, other coronaviruses like HKU1, NL63, OC43, and 229E cause mild symptoms [16]. Virion particles enter the host cell *via* binding through the ACE2. Subsequently, its genome (ss RNA) gets attached to the host's ribosomes, resulting in the translation of 2 *co*-terminal and large polyproteins that are further processed by proteolytic enzymes/proteolysis [13,17]. A brief outline of the virus life cycle is illustrated in Fig. 1. Proteolysis mediated by 3CLpro and PLpro [18,19], slices large polyproteins into smaller components for the folding and packaging of new virions, which consequently promotes the spread of infection. The main protease is a key enzyme of SARS-CoV-2, which plays a pivotal role in mediating viral replication and transcription, making it an attractive drug target [20]. Another interesting enzyme, RdRp, a replicase is essential for the replication of the viral genome [21].

These major enzymes, 3CLpro, PLpro, and RdRp are responsible for the proteolysis, replication, and production of new virions [22,23]. Hence, these enzymes are considered as potential drug targets for the development of therapeutic strategies, as they are crucial for the survival, replication, and transmission of the viruses. In this viewpoint, we investigated the sequence similarities of these proteins with their close viruses like SARS-CoV (2002) and MERS-CoV (2013) for which possible drugs and vaccines were developed. A deeper understanding of sequence similarity and differences in the conserved region might help to design improved therapeutics approaches.

Generally, catalytic pockets of potential target enzymes are the important regions for the design and development of small-molecule inhibitors. We observed that pocket residues of these enzymes are highly conserved, and share sequence similarity with the corresponding CoVs. Since the SARS-CoV-2 shares a high sequence identity in their RdRp and 3CLpro proteins to the SARS-CoV and MERS-CoV. Therefore, it is believed that therapeutic molecules used for targeting the SARS-CoV and MERS-CoV could be useful for the treatment of COVID-19 with a similar efficacy. However, SARS-CoV-2 spike RBD is significantly different from the SARS-CoV, especially in the two regions that interact with ACE2. This difference made the previously developed antibodies and therapeutic peptides for the SARS-CoV spike RBD not to work efficiently against SARS-CoV-2 and thus demands newer structure-based drugs and therapies developed by considering these structural differences [24].

CoVs influence the host cells by its cytocidal activity as well as by immune-mediated mechanisms [25]. Many studies revealed that infection of CoVs leads to cytopathic effects, including apoptosis and cell lysis. The virus causes cellular fusion leading to the formation of syncytia. The above-mentioned processes are witnessed in the cell due to the mobilization of vesicles to form the replication complex and disruption of Golgi complexes during viral replication. Compared to other CoVs like the SARS-CoV and MERS-CoV have shown to exert their cytopathic effects in the kidney cells and the formation of syncytia in lung tissues.

SARS-CoV-2 pathogenesis involves innate as well as an adaptive immune system [26]. During viral infection, T cells and secondary messengers like cytokines play important roles in the progression of the disease. Evolutionarily conserved innate pattern recognition receptors (PRRs) recognize molecules associated with pathogens, or released by damaged cells are found to be involved in modulating the innate and adaptive immune response to SARS-CoV-2 infection. Besides T cells, humoral antibodies have been reported to be crucial in infections caused by CoVs.

3. Genome structure

The genome of SARS-CoV-2 is comprised of a single-stranded positive-sense RNA [27]. The newly sequenced genome of the SARS-CoV-2

was submitted in the NCBI genome database (NC_045512.2) ~29.9 Kb in size [11]. The genetic makeup of SARS-CoV-2 is composed of 13–15 (12 functional) open reading frames (ORFs) containing ~30,000 nucleotides. The genome contains 38% of the GC content and 11 protein-coding genes, with 12 expressed proteins. The genetic arrangement of ORFs highly resembles the SARS-CoV and MERS-CoV [28,29]. The ORFs are arranged as replicase and protease (1a–1b) and major S, E, M, and N proteins, which follows a typical 5′-3′ order of appearance, and are considered as major drug/vaccine targets. These gene products play important roles in viral entry, fusion, and survival in host cells [23].

The genomic organization of the SARS-CoV-2 is sharing about 89% sequence identity with other CoVs (Fig. 2A). The translated sequences of SARS-CoV-2 proteins were retrieved from the GenBank (Accession ID: NC_045512.2)]. The whole genome of SARS-CoV-2 encodes about 7096 residues long polyprotein which consists of many structural and non-structural proteins (NSPs). The nucleotide content of the viral genome is held mainly by two non-structural proteins ORF1a and ORF1ab followed by structural proteins. Polyproteins pp1a and pp1ab are encoded by ORFs 1a and 1b, where polyprotein pp1ab is encoded by the ribosomal frameshift mechanism of the gene 1b. These polyproteins are further processed by virally encoded proteinases and produce 16 proteins, which are well conserved in all CoVs belonging to the same family (Fig. 2B).

MERS-CoV is closely related to the SARS-CoV-2 as it carries a larger genome with ~30,119 nucleotides. The genetic makeup of MERS contains a 5′ cap structure, a poly(A) tail at 3′ end, the *rep* gene containing 16 NSPs which were numbered as nsp1-nsp16 from the 5′ end. Around 10 kb of the genome at 3′ end constitutes 4 structural genes (S, E, M, N) and 5 accessory proteins (ORF3, ORF4a, ORF4b, ORF5, ORF8). The SARS-CoV-2 is relatively more infectious in comparison to the SARS-CoV and MERS-CoV possibly due to different epidemiological dynamics. It may be possible that other mammalian species act as an “intermediate” or “amplifying” hosts and subsequent ecological separation acquired some or all of the mutations needed for efficient human transmission [30].

Comparative sequence analysis of the SARS-CoV-2 genome indicates striking similarities to the BAT-CoV, suggesting a possible mammalian origin from bats in the Wuhan city of China [31]. However, there is evidence suggesting bats as the natural reservoirs of SARS-like CoVs, including the SARS-CoV-2 [32–34]. CoVs needed intermediate hosts before transmitted to humans. A probable Pangolin origin of SARS-CoV-2 was also suggested, based on the significant similarity of the certain gene [35]. It is still not clear how the bat CoVs are genetically transformed and reached to humans? Another evidence suggested that dogs get infected by SARS-CoV-2. It is interesting to note that angiotensin-converting enzyme (ACE2) of both humans and dogs share high sequence identity (13 out of 18) and thus their binding to the spike RBD of SARS-CoV-2 are quite similar, suggesting human-to-animal transmission [36].

4. Non-structural proteins

In addition to the capsid-forming structural proteins, the viral genome encodes many NSPs that perform numerous roles in the replication and virus assembly processes [37]. These proteins participate in viral pathogenesis by modulating early transcription regulation, helicase activity, immunomodulation, gene transactivation, and countering the antiviral response [38–40].

We explored some of the major functions of NSPs in SARS-CoV-2 (Table 1). The InterProScan search revealed that NSPs of SARS-CoV-2 are involved in many biological processes including, viral genome replication (GO:0019079 and GO:0039694), protein processing (GO:0019082), transcription (GO:0006351), and proteolysis (GO:0006508). These proteins are involved in the RNA-binding (GO:0003723), endopeptidase activity (GO:0004197), transferase activity (GO:0016740), ATP-binding (GO:0005524), zinc ion binding (GO:0008270), RNA-directed 5′-3′ RNA-polymerase activity (GO:0003968), exoribonuclease activity, producing 5′-phosphomonoesters (GO:0016896), and methyltransferase activity (GO:0008168).

To explore the intrinsically unstructured regions in SARS-CoV-2 polyprotein, the translated sequence of SARS-CoV-2 ORF1ab polyprotein was retrieved from the GenBank (Accession ID: NC_045512.2). We have predicted the intrinsically unstructured regions in SARS-CoV-2 polyprotein through multiple predictors such as PONDR® (Predictor of Natural Disordered Regions), VLXT, VL3, VLS2 [55] and IUPred2A web servers [56]. These tools allowed us to identify disordered protein regions by predicting the residues which do not possess the tendency to form a structure in the native condition. Residues with a score of > 0.5 thresholds were considered to be intrinsically disordered; whereas, residues with a score between 0.2 and 0.5 were considered as flexible. The graph shows the disordering tendency of each residue in the SARS-CoV-2 polyprotein, where higher values correspond to a higher probability of disorder (Fig. 3). The data analysis suggests that the SARS-CoV-2 has a chunk of intrinsically disordered regions that lack a well-defined tertiary structure under native conditions. The N-terminal region of Nsp3 (920–1020) shows a higher tendency to be disordered as predicted by all four predictors. Further, this analysis provides a brief insight into the non-structural proteome as well as the unstructured protein regions of the SARS-CoV-2 polyprotein that may be useful to understand the structural basis of infection, structure-based drug discovery and interaction of SARS-CoV-2 proteins with host proteins in different physiological conditions.

5. Potential drug targets

The small machinery of proteins of human CoVs as discussed in the preceding section contributes to the pathogenesis by helping the virus entry into the host cell (Fig. 1). We observed that the spike glycoprotein (S), an envelope protein (E), membrane protein (M), nucleoprotein (N), and two isoforms of replicase polyprotein, namely 1a and 1ab are considered as potential drug/vaccine targets. Replicase polyproteins contain the main protease domain, including 1ab with the RNA-binding RdRp domain that plays crucial roles in the process of pathogenesis.

We performed sequence analysis of these proposed drug target proteins using multiple sequence alignment (MSA) to understand the relationship among viruses in terms of sequence conservation and divergence. A BLASTp search indicated that out of 12 predicted coding regions (1ab, S, 3, E, M, 7, 8, 9, 10b, N, 13 and 14), most of them are similar in the length to the bat-SL-CoVZC45 and bat-SL-CoVZXC21 with a few insertion and deletion except for a longer spike protein. Large number of proteins showed a high level of sequence similarity between SARS-CoV-2 and CoV of bat-origin, with a 90% sequence identity for 1a, 86% for 1ab but a lesser sequence identity for spike protein (~ 80%) and protein 13 (73.2%). Protein 1ab is quite similar between SARS-CoV-2 and SARS-CoV, while 3a and 8b accessory genes of SARS-CoV-2 are closest to the SARS-CoVs. However, the RdRp is quite distinct among them [57].

A systematic comparison showed significant differences in the proteins of human-infecting CoVs: 8a protein present in SARS-CoV but absent in SARS-CoV-2; and 8b protein is longer in SARS-CoV-2 (121 aa). A total of 380 amino acid substitutions were identified between SARS-CoV-2 and consensus sequences of SARS and SARS-like CoVs, possesses divergence in functional and pathogenic characters of SARS-CoV-2. These substitutions were observed mainly in the nsp3, nsp2, and S protein; whereas, nsp7, nsp13, envelope, matrix, or accessory proteins p6 and 8b were conserved. Based on protein sequence similarity, it can be inferred that three viral species, namely, SARS-CoV, bat-SL-CoVZC45, and bat-SL-CoVZXC21 are very similar to SARS-CoV-2. Here, we discuss the roles and sequence comparison of each drug target among four strains of coronaviruses, *i.e.*, BAT-CoV HKU3, SARS-CoV, MERS-CoV, and SARS-CoV-2 strains.

Table 1
List of non-structural proteins in SARS-CoV-2 and their molecular functions.

S. No.	Range	Protein name and ID	Description	Proposed function
1.	1–180	Nsp1 YP_009725297.1	Nsp1 is the N-terminal product of the viral replicase	Leader protein host translation inhibitor. Mediates RNA replication and processing. Involved in mRNA degradation [41].
2.	181–818	Nsp2 YP_009725298.1	Nsp2 is a replicase product essential for proofreading viral replication	Modulation of host cell survival signalling pathway by interacting with host PHB and PHB2 [42].
3.	819–2763	Nsp3 YP_009725299.1	Nsp3 is a papain-like proteinase contains several domains.	Functions as a protease to separate the translated polyprotein into its distinct proteins [43,44].
4.	2764–3263	Nsp4 YP_009725300.1	A membrane-spanning protein contains transmembrane domain 2 (TM2)	Believed to anchor the viral replication-transcription complex to modified ER membranes [45].
5.	3264–3569	Nsp5 YP_009725301.1	3C-like proteinase and main proteinase	Involved in viral polyprotein processing during replication [46].
6.	3570–3859	Nsp6 YP_009725302.1	Putative transmembrane domain	Plays a role in the initial induction of autophagosomes from host endoplasmic reticulum.
7.	3860–3942	Nsp7 YP_009725303.1	Nsp7 is an RNA-dependent RNA polymerase	It forms a hexadecameric super-complex with nsp8 that adopts a hollow cylinder-like structure implicated replication [47,48].
8.	3943–4140	Nsp8 YP_009725304.1	Multimeric RNA polymerase; replicase	It forms a hexadeca-meric super-complex with nsp7 that adopts a hollow cylinder-like structure implicated replication [47,48].
9.	4141–4253	Nsp9 YP_009725305.1	A single-stranded RNA-binding viral protein	Participate in viral replication by acting as an ssRNA-binding protein [49].
10.	4254–4392	Nsp10 YP_009725306.1	Growth-factor-like protein contains two zinc-binding motifs	In viral transcription by stimulating both nsp14 3′-5′ exoribonuclease and nsp16 2′-*O*-methyltransferase activities. Therefore plays an essential role in viral mRNAs cap methylation [50].
11.	4393–5324	Nsp12 YP_009725307.1	RNA-dependent RNA polymerase (Pol/RdRp)	Responsible for replication and transcription of the viral RNA genome [51].
12.	5325–5925	Nsp13 YP_009725308.1	Zinc-binding domain, NTPase/helicase domain, RNA 5′-triphosphatase	A helicase core domain that binds ATP. Zinc-binding domain is involved in replication and transcription [52,53].
13.	5926–6452	Nsp14 YP_009725309.1	Proofreading Exoribonuclease domain (ExoN/nsp14)	Exoribonuclease activity acting in a 3′ to 5′ direction and N7-guanine methyltransferase activity.
14.	6453–6798	Nsp15 YP_009725310.1	EndoRNAse; nsp15-A1 and nsp15B-NendoU	Mn(2+)-dependent endoribonuclease activity
15.	6799–7096	Nsp16 YP_009725311.1	2′-O-ribose methyltransferase	Methyltransferase that mediates mRNA cap 2′-O-ribose methylation to the 5′-cap structure of viral mRNAs [54].
16.	4393-4405	Nsp11 YP_009725312.1	Made of 13 amino acids (sadaqsflngfav) and identical to the first segment of Nsp12.	Unknown

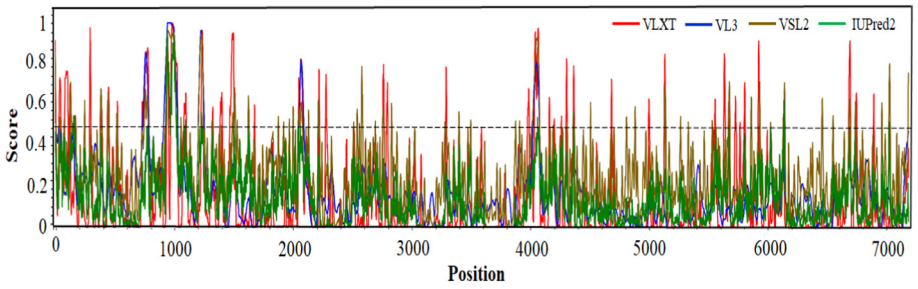

Fig. 3. Graph illustrating the disordering tendency of each residue in SARS-CoV2 polyprotein. The dotted line is the threshold value of 0.5.

5.1. Spike Glycoprotein (S)

Spike glycoprotein plays a significant role in pathogenesis by binding to the host cell through its RBD [58]. The S protein initiates the infection by sticking the virion to the host cell. It is composed of 1273 amino acid residues containing three subunits, namely S1, S2, and S2' which act differently during the process of adherence to the host cell. The S1 subunit is involved in the attachment of virions with the host cell membrane by interacting with human ACE2 that subsequently initiates the infection process [17]. During this process, S protein undergoes conformational changes induced upon its entry into the endosomes of the host cell [24]. The understanding of these conformational changes is essential for the process of vaccine development as dynamic changes in the target protein might affect immune responses [59]. Mutations in the S protein seem to induce conformational changes, which may cause an altered antigenicity. Although several mutations have been found in the S1 receptor binding region of SARS-CoV-2, its interaction with ACE2 is preserved in humans, swine, civet, and bats, except for mouse ACE2 [60–62].

The other subunit of the S protein, S2 works as the fusion protein that helps in the fusion of virion with the mammalian cell membrane. During the process of fusion, the S2 protein appears in three main conformational states 1) pre-fusion native state, followed by 2) a pre-hairpin intermediate state, and 3) ensuing post-fusion hairpin state. It is interesting to understand how these dynamic conformation states orchestrate the mechanism of viral entry into the host cell membrane for this might lead to the development of effective therapeutics [59]. The remaining S2' cleaved subunit of the S protein functions as a fusion peptide [63]. The sequence of spike stalk S2 of SARS-CoV-2 is highly similar to bat SARS-like CoVs and human SARS-CoV (~99%), indicating a broad spectrum use of antiviral compounds designed against S2 domain of these viruses, which may be useful in COVID-19 therapy [15,64].

The RBD of spike protein is the most variable part of the SARS-CoV-2. MSA profile of the RBD of S protein suggested that three CoVs (BAT-CoV HKU3, SARS-CoV, and SARS-CoV-2) share a typical evolutionary pattern, while MERS-CoV has a different RBD amino acid composition showing a divergence from the previously mentioned strains of the virus (Fig. 4A). The ~90 amino acid long receptor binding motif of the RBD facilitates the binding of the virus to the host receptor shows the

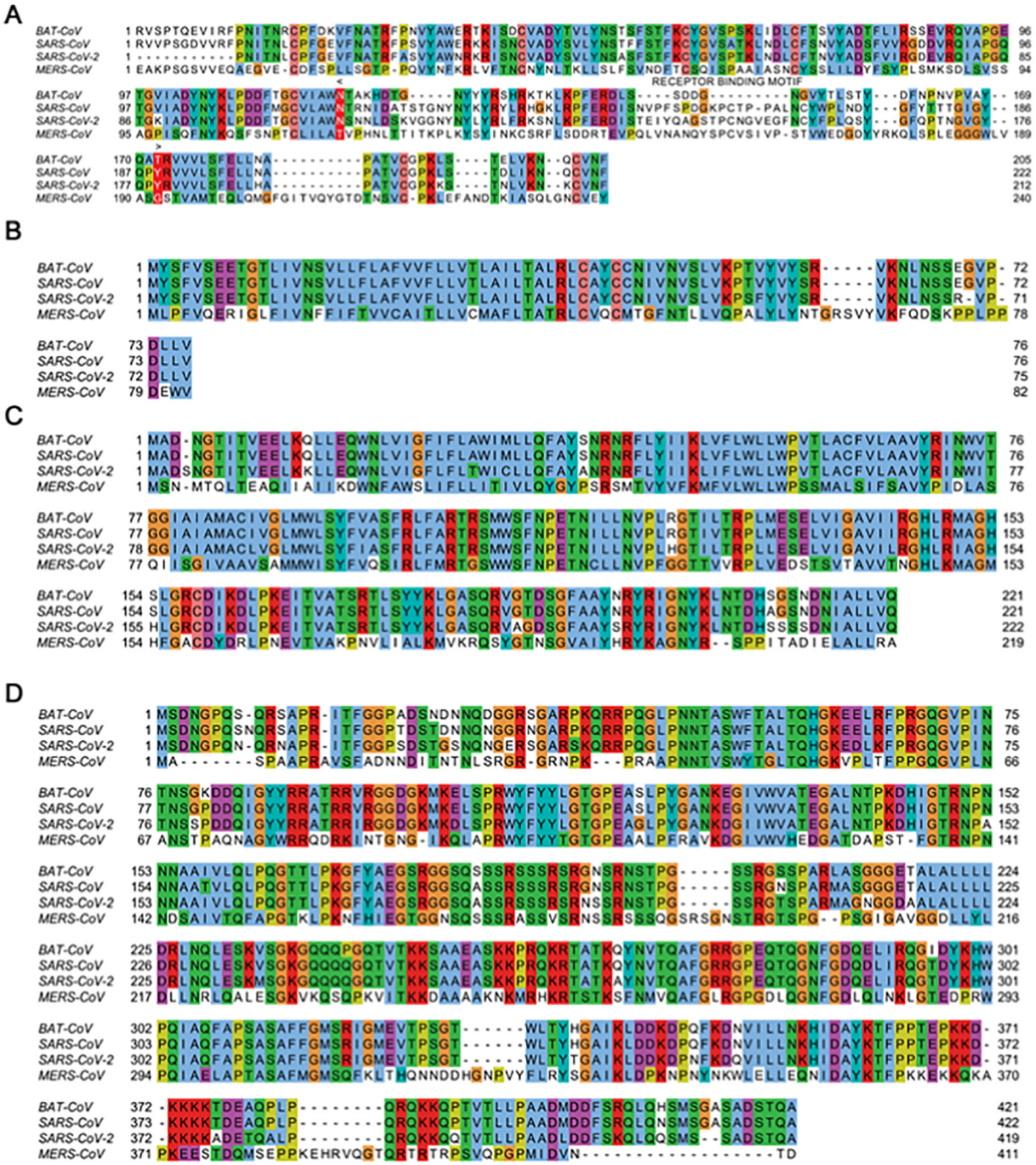

Fig. 4. Multiple sequence alignment of A. RBD of the spike glycoprotein, **B.** Envelope protein, **C.** Membrane protein, **D.** MSA of nucleoprotein showing regions of sequence conservation.

least conservation, suggesting the involvement of multiple mechanisms in pathogenesis. After further analysis, we identified six residues of RBD of SARS-CoV2 (Leu455, Phe486, Gln493, Ser494, Asn501, and Tyr505), that are critical for binding to ACE 2, five differ from SARS-CoV, a feature that needs to be factored in drug design and development [16].

5.2. Envelope protein (E)

Envelope membrane (E) proteins are a group of relatively small viral proteins that help in the assembly and release of the virions [65]. Among the structural proteins of the SARS-CoV-2, E protein is considered as a potential drug target. The E protein is relatively small (75 aa), and plays a significant role in the viral morphogenesis and assembly [66]. The E protein is known to act as viroporins that assemble into host membrane forming protein-lipid pores involved in ion transport. The sequences of E protein for all four strains are highly conserved regions among the BAT-CoV, SARS-CoV, and SARS-CoV-2 while exhibiting a slight variation in the sequence of MERS-CoV envelope proteins (Fig. 4B).

5.3. Membrane protein (M)

M proteins are 222 amino acid long structural proteins that function in concurrence with E, N, and S proteins, and plays a major role in the RNA packaging [67]. The conserved stretch of amino acids suggests a common architecture for these proteins (Fig. 4C). M proteins are the

most abundant viral proteins of CoVs that are involved in providing distinct shape to the virus. MSA profile of the M protein shows a higher sequence conservation among BAT-CoV, SARS-CoV, and SARS-CoV-2 (Fig. 2B). However, a considerable variation in the sequence of the M protein of the MERS-CoV strain was observed. The presence of three transmembrane domains is a distinct feature of M proteins.

5.4. Nucleoprotein (N)

Nucleocapsid proteins (N) play an important role in the packaging of viral RNA into ribonucleocapsid [68]. N protein of SARS-CoV-2 is highly conserved across CoVs sharing ~90% sequence identity with that of SARS-CoV. It mediates viral assembly by interacting with the viral genome and M protein, which are helpful in the augmentation of viral RNA transcription and replication [69]. Thus, N proteins are considered as potential drug targets. The N proteins bind to viral RNA through its ~140 amino acid long RNA-binding domain in their core in a "bead on a string" manner [65]. MSA profile of N protein from the BAT-CoV, SARS-CoV, and SARS-CoV-2 show highly conserved regions (Fig. 4D). Based on the high sequence similarity of N protein, it may be suggested that antibodies against the N protein of SARS-CoV would likely to recognize the N protein of SARS-CoV-2. A similar pattern has been observed for the MERS-CoV strain, where regions of slight sequence variations suggesting its divergence in the evolutionary process.

5.5. Replicase polyprotein

Replicase polyprotein is another essential enzyme that helps in the cleavage of host RNA and replication of the viral RNA [21]. As discussed earlier, the non-structural ORFs 1a and 1ab share the majority of nucleotide content of the viral genome. Replicase polyproteins are multifunctional proteins that perform various tasks, contributing to the viral pathogenesis [70]. However, the principal role of these proteins is to help in the transcription and replication of the viral RNA. Mainly, these proteins are subdivided into various NSPs such as nsp1, nsp2, nsp3, nsp4, and proteases such as PLpro and 3CLpro. The ORF1ab contains a specific RdRp domain playing a pivotal role in the viral RNA transcription and replication.

The replicase polyprotein is composed of three domains including, macro-, papain-like- and the main protease domains. MSA of replicase polyprotein 1a shows a conserved region among all three domains except for the main protease where regions of least sequence conservation are present (Fig. 5A-C). The main protease of SARS-CoV, and SARS-CoV-2 show higher sequence similarity (~96%) suggesting a common evolution. The main protease domain of MERS-CoV does not show any similarity with the other three viral strains, suggesting a clear divergence in the evolution. Replicase polyprotein 1ab also contains macro, papain-like, and main protease domains with an addition to the RdRp catalytic domain which acts as an RNA-binding polyprotein. The MSA of replicase polyprotein 1ab suggests higher sequence conservation among all four strains of CoVs (Fig. 5D-G).

6. Structure analysis and comparison of target proteins

The detailed knowledge of the structural organization of the target proteins is helpful in drug and vaccine development for therapeutic management of COVID-19 [71]. This section deals with the structural analysis of the target proteins of SARS-CoV-2 and compared to other known human CoVs. For the ease of visualization, graphical representation of the target proteins and their subsequent domains is provided in the supplementary section. A broader picture of the structural similarities and the variations can be viewed in terms of MSAs discussed in the previous section. However, an in-depth description of the structure of target proteins at the active/binding site shows a larger variation.

The S protein is one of the most important drug/vaccine targets in CoV pathogenesis because the RBD of the S1 domain undergoes a hinge-like conformational movement and an important determinant of host cell receptor binding [7]. Such interactions help the virus to stick to the host cell ACE2 by the RBD [22,72]. Because of the vital function of the S protein, it is considered as an attractive target for antibody-mediated vaccine and drug development [62,73]. The overall structure of the S protein of SARS-CoV-2 closely resembles with the SARS-CoV (RMSD = 3.8 Å for 959 C^{α} atoms). However, the position of the RBD was found relatively in down conformation. This difference is evident as in the case of SARS-CoV RBD in the down conformation packs tightly against the N-terminal domain of neighbouring protomer. The receptor-binding motif of the S protein shows a notable structural difference and thus small molecule inhibitors designed against other CoV strains seem ineffective (Fig. 6, S1). On the other hand, the RBD in the down conformation of SARS-CoV-2is angled closer to the central cavity of the trimer. Despite this little conformational difference, structural domains of SARS-CoV-2 S protein shares a high degree of structural similarity to their counterparts from SARS-CoV S protein with calculated RMSD for the NTDs, RBDs, subdomains 1 and 2 (SD1 and SD2), and S2 subunits as 2.6 Å, 3.0 Å, 2.7 Å, and 2.0 Å, respectively [61].

Despite a high sequence similarity (~98%) of SARS-CoV-2 S protein to the bat CoV RaTG13, insertion of the S1/S2 protease cleavage site is observed in the case of SARS-CoV-2, resulted in an "RRAR" furin recognition site. Insertion of such a polybasic furin site is the possible reason for the high degree of virulence [74]. Structure analysis revealed that the S1/S2 junction is present in a disordered, solvent-exposed loop which is not much conserved in other CoVs, causes a substantial effect on the structure or function [61]. RBD-directed monoclonal antibodies of SARS-CoV sharing common three epitopes were tested for SARS-CoV-2, shows insignificant binding. Hence, a comparative structure analysis will enable the design and screening of a new class of small-molecule inhibitors which can interfere with the host fusion. Further, structural information will support to design a precise vaccine to accelerate therapeutic measures against COVID-19.

No significant difference was observed in the architecture of the E proteins (Fig. S2). M protein does not show any significant difference in protein architecture (Fig. S3). Similarly, nucleoprotein also shows a higher similarity in the core RNA-binding domain of the N protein (Fig. S4). Both isoforms of replicase polyprotein 1a and 1ab show significant structural similarities except for the main protease of the 1a isoform where it shows structural differences with those of the other three strains when compared (Fig. S5 and S6). These observations are in agreement with the results of MSA.

RdRp domain present in the 1ab isoform of the replicase polyprotein shows a significant structural similarity among all four strains (Figs. S7–S12). The polymerase domain of RdRp adopts a similar architecture of the viral polymerase family as it is composed of a finger, a palm, and thumb subdomains. However, the conserved catalytic metal ion is absent due to the lack of primer-template RNA and NTPs. The catalytic site of the RdRp domain of SARS-COV-2 is formed by the conserved polymerase motifs A-G in the palm domain and configured like other RNA polymerases. Motif A (611-TPHLMGW**D**YPKCDRAM-626), Motif C (753-FSMMIL**SDD**AVVCFN-767), and the catalytic residues (759-**SDD**-761) are conserved in most viral polymerases [75].

The main protease is one of the most characterized drug targets of the CoVs as this enzyme is essential for processing the polyproteins required for viral assembly [76]. Despite a very high sequence identity (96%) between the SARS-CoV and SARS-CoV-2, they differ at 12 positions (residues number 33, 44, 63, 84 86, 92, 132, 178, 200, 265, 283 and 284) in the sequence which leads an RMSD value of 0.53 Å for all C^{α} atoms by comparing their published structures [77,78]. We observed that the main protease of replicase polyprotein 1ab isoform shows significant similarities when compared between other related strains (Fig. 7).

The observations in the preceding sections helped to deduce the intricate mechanisms adopted by the viral strains in terms of

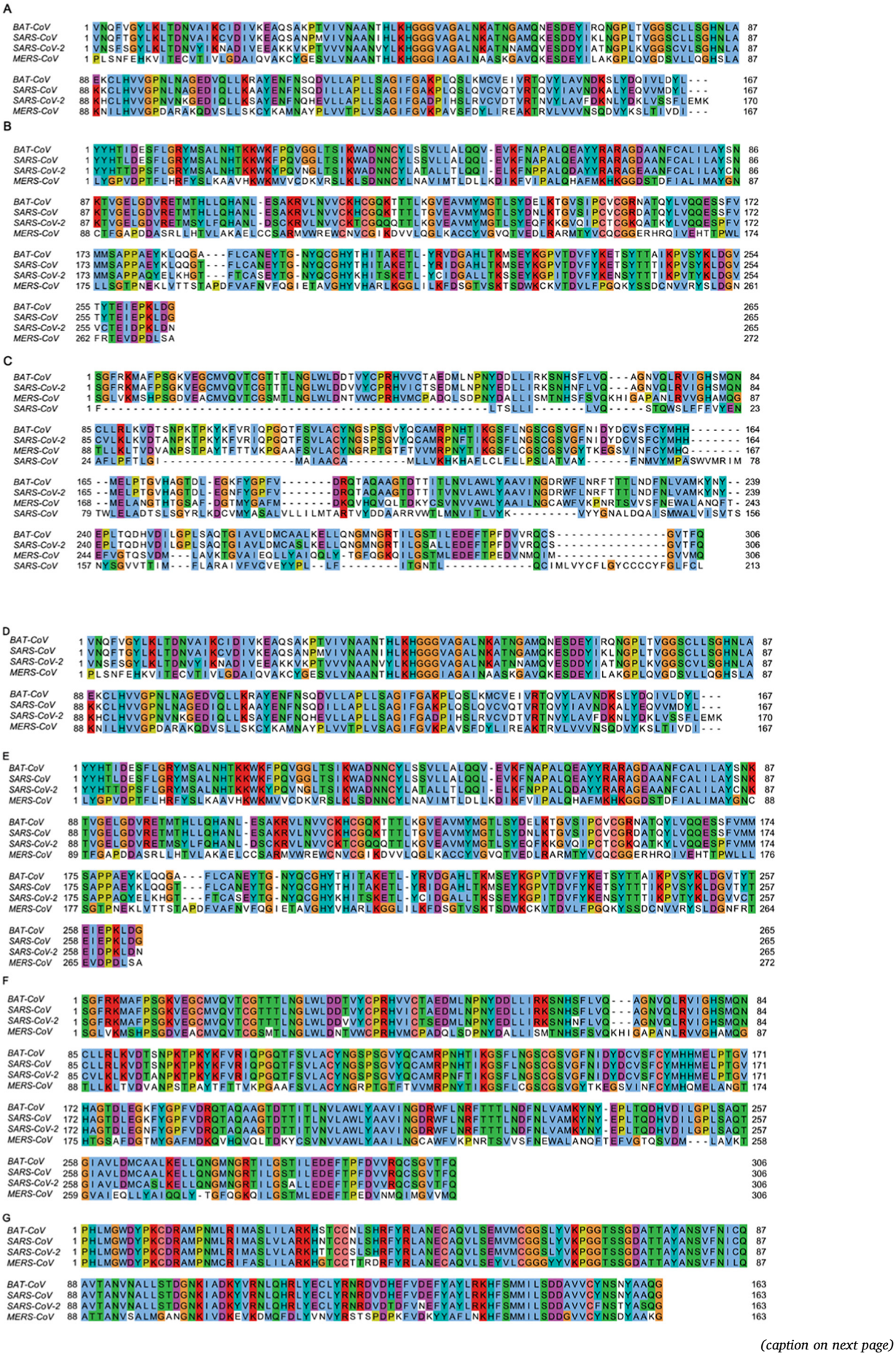

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Fig. 5. MSA of the Replicase polyprotein 1a and 1ab showing sequence conservation in macrodomains. **A.** Papain like domain, **B.** Main protease, **C.** Highly dissimilar, and flanking regions. **D.** The macro domain of Replicase polyprotein 1ab, E. Papain like domain, F. Main protease, and G. RdRp domain.

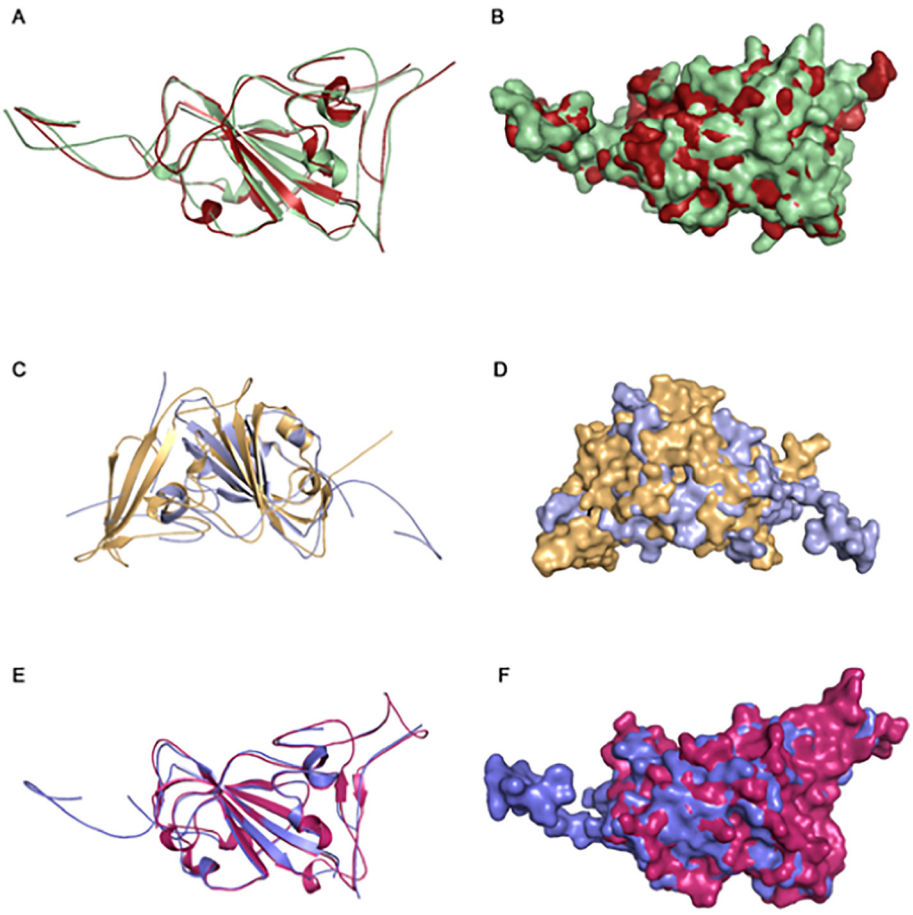

Fig. 6. Structural comparison of RBDs of S protein for all four strains. **A.** Superposed image of BAT CoV S RBD protein (lime green) and SARS-CoV-2 (red) (RMSD: 2.3 Å), **B.** Surface representation of superimposed RBD of BAT-CoV and SARS-CoV-2. **C.** Superposed image of RBD of MERS-CoV S protein (light orange) and SARS-CoV-2 (light blue) (RMSD: 8.6 Å), **D.** Surface representation of the RBD of MERS-CoV and SARS-CoV-2, **E.** Superposed image of RBD of SARS-CoV S protein (warm pink) and SARS-CoV-2 (slate) (RMSD: 1.5 Å), **F.** Surface representation of the RBD of SARS-CoV and SARS-CoV-2.

pathogenesis and drug resistance. These explanations are also accommodating in setting up the therapeutic strategies against these target proteins. We discuss these aspects by taking the example of the receptor-binding motif of the S protein. As discussed, it contains the most structurally diverse regions as compared to other target proteins. Due to its significance in the pathogenesis, the S protein is considered as a target for drug development against coronavirus infections. Hence, it is interesting to know that structural variations might have a significant role in drug resistance.

7. Inflammatory pathways and cytokine response

The innate immune response forms the first line of defense against viral infections. However, when the immune response is dysregulated, it will result in excessive inflammation, and even death [79]. During the CoV infections, the innate immune responses have been involved in driving a cytokine storm and altering the adaptive immune responses [80]. CoVs are RNA viruses that are recognized by intracellular pattern recognition receptors. This recognition leads to the activation of signalling cascades, culminating in the release of cytokines and chemokines, which directs the recruitment of immune cells to the site of infection [81]. These immune cells, based on their activation status are involved in the clearance of pathogen using various mechanisms.

Here, we compare the inflammatory pathways and cytokine responses during SARS-CoV, MERS-CoV, and SARS-CoV-2 infections [82]. The ORF8a and ORF9b trigger cellular apoptosis. ORF8b induces DNA synthesis and suppressing viral envelope protein expression. ORF7a activates nuclear factor-κB (NF-κB) and ORF6 limits interferon production, while ORF3a induces necrotic cell death. ORF9b alters interferon responses by promoting the degradation of mitochondrial antiviral signalling protein [83]. Overall, SARS-CoV ORFs engage multiple pathways that control disease severity. Further, *in vitro* studies of SARS-CoV infection of macrophages, dendritic cells, and epithelial cell lines, showed low levels of type I interferon production similar to *in vivo* responses observed in the mice and humans [84].

In the case of SARS-CoV and MERS-CoV, both serine protease 2 and translation elongation factor 1 (EF-1A) of the host strongly bind to N protein and subsequently induces local or systemic inflammatory responses. The N protein of MERS-CoV binds to the E3 ubiquitin ligase of triple motif protein 25, preventing the interaction between the triple motif protein 25 and retinoic acid-inducible gene I. Blocking the ubiquitination and activation of the retinoic acid-inducible gene I mediated by triple motif protein 25 ultimately leads to the inhibition of type-I IFN production, suggesting that the N protein of CoV regulates the host's immune response against the virus. Human cell culture models of MERS infection have shown a deficiency in interferon induction and innate immune responses, which may result in minor evolutionarily difference in MERS-CoV as compared to other CoVs, and engagement of distinct mechanisms of regulation of host antiviral responses [85]. Other virus molecules, in addition to accessory protein 4a (p4a), the viral PLpro also blocks IFN-β induction, as well as downregulate the expression of *CCL5* and *CXCL10* pro-inflammatory cytokine genes [86,87]. A transcriptomic approach revealed the infection of human lung epithelial cell line with MERS-CoV and SARS-CoV induced similar pathogen recognition receptor genes and pro-inflammatory cytokine genes related to interleukin 17 (IL-17) signalling by IL-17A and IL-17 F

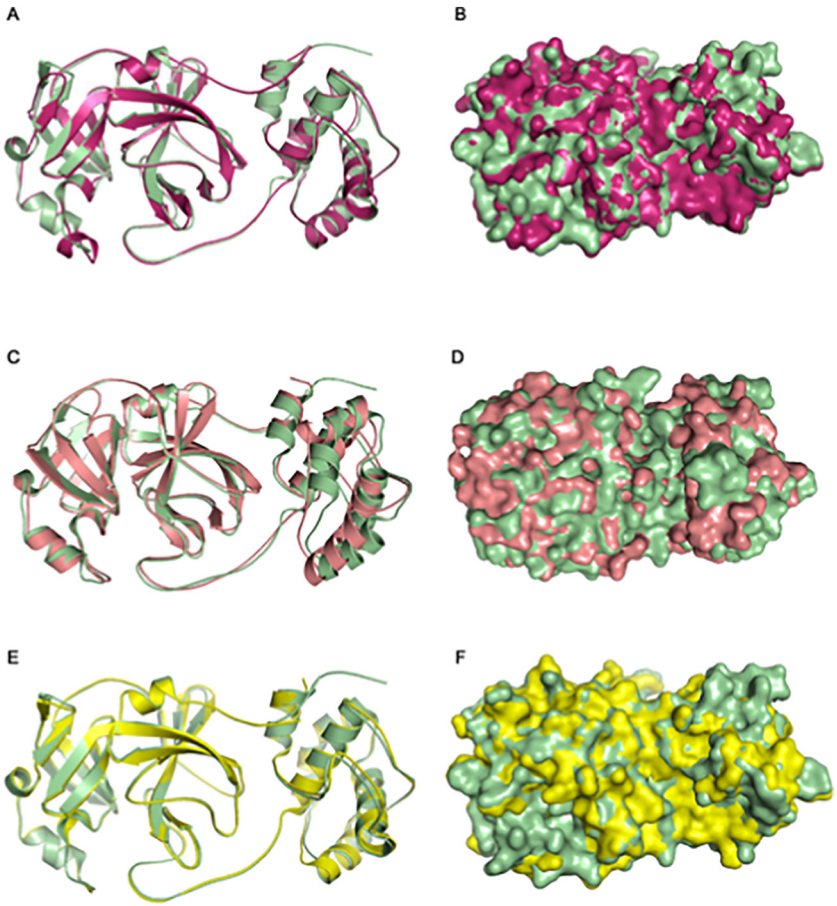

Fig. 7. Structural comparison of Replicase polyprotein 1ab main protease for all four strains. **A.** Superposed image, and **B.** Surface representation of the main protease of BAT-CoV (warm pink) and SARS-CoV-2 (pale green) (RMSD: 1.9 Å). **C.** Superposed image, and **D.** Surface representation of main protease MERS-CoV (salmon) and SARS-CoV-2 (pale green) (RMSD: 2.7 Å), **E.** Superposed image and **F.** Surface representation of the main protease of SARS-CoV (yellow) and SARS-CoV-2 (pale green) (RMSD: 1.1 Å).

cytokines, but MERS-CoV infection downregulates the genes involved in antigen presentation pathway [88].

SARS-CoV-2 infection also resulted in cytokine dysregulation similar to SARS-CoV and MERS-CoV [89], as evident by the presence of abnormally low levels of antiviral cytokines. Patients infected with SARS-CoV-2 show high levels of pro-inflammatory cytokines including IL-1, IL-2, IL-6, IL8, IL-17, G-CSF, GM-CSF and chemokines such as IP-10 and MCP-1 in the sera during the disease, and may play a key role in the development of lung dysfunction by leading to the accumulation of immune cells within the lungs [90–93]. Increased concentrations of IL-6 are associated with increased viral load and the recruitment of inflammatory monocytes [94]. Suppressor of cytokine signalling 3 (SOCS3) regulates the negative feedback mechanism of IL-6, which is found to be reduced in the patients with COVID-19 [95]. Plasma TNF-α was found to be moderately regulated in SARS-CoV patients [96]. In summary, the three viral infections have marginal difference in terms of innate immune responses but greatly differ in terms of morbidity and mortality.

8. Therapeutics approaches

Therapies available for CoVs are mainly divided into either acting on targets of the human immune system or human cells, or another one is the virus itself. Fig. 8 shows the target sites for different drugs in the life cycle of the virus. The major attention of the scientific community has now shifted towards the SARS-CoV-2, leaving various essential projects in limbo [97]. The identification of available antiviral drugs as potential candidates through drug repurposing [98,99], state of the art *in silico* methods to discover novel drugs [100,101], allowing the use of anti-inflammatory drugs for treating COVID-19 [102]. As we have discussed, subtle structural differences in the target proteins lead to possible hurdles in the process of identifying effective therapeutics using the aforementioned strategies.

Management of COVID-19 patients has been symptomatic approach, and the severe cases are provided with ventilation assistance. Prevention is achieved by propagating the importance of regular hand washing, avoiding touching of the face, and adopting social distancing where individuals are asked to maintain one-meter distance from each other. Also, several drug molecules have been tried in the last couple of months as a treatment strategy. Some of the studies relating to the possible treatment of COVID-19 has been highlighted in Table 2.

The existing broad-spectrum antiviral drugs used to treat pneumonia-like symptoms, interferons, ribavirin, and cyclophilin are the first line of the therapeutic option [115]. Remdesivir, an analog of adenosine, seems to have a more promising future. Remdesivir is an adenosine analog terminates the synthesis of viral RNA chains by incorporating in place of real nucleotide. In a recent study, it was shown that remdesivir binds to RdRp and inhibits its activity [75]. This drug has been effective against single-stranded RNA viruses including MERS and SARS-CoV. Encouraged by these results, remdesivir is being advocated for the treatment of SARS-CoV-2 [104]. Remdesivir, as a single agent drug, was used for individual cases of COVID-19 in Italy and the Czech Republic, in March 2020. But the outcomes of these trials still need to be verified before it is declared as being successful. Remdesivir along with chloroquine effectively inhibited SARS-CoV-2 *in vitro*. Further studies and clinical trials in humans will be required before it is declared as being effective against COVID-19 [116].

In the frantic search for drug molecules against SARS-CoV-2, repurposing of antimalarials drugs for COVID-19 shown some positive impact [117]. Among these, chloroquine has gained a lot of attention in the last few months as an option to treat COVID-19 [118,119]. Chloroquine has antiviral effects by increasing endosomal and lysosomal pH

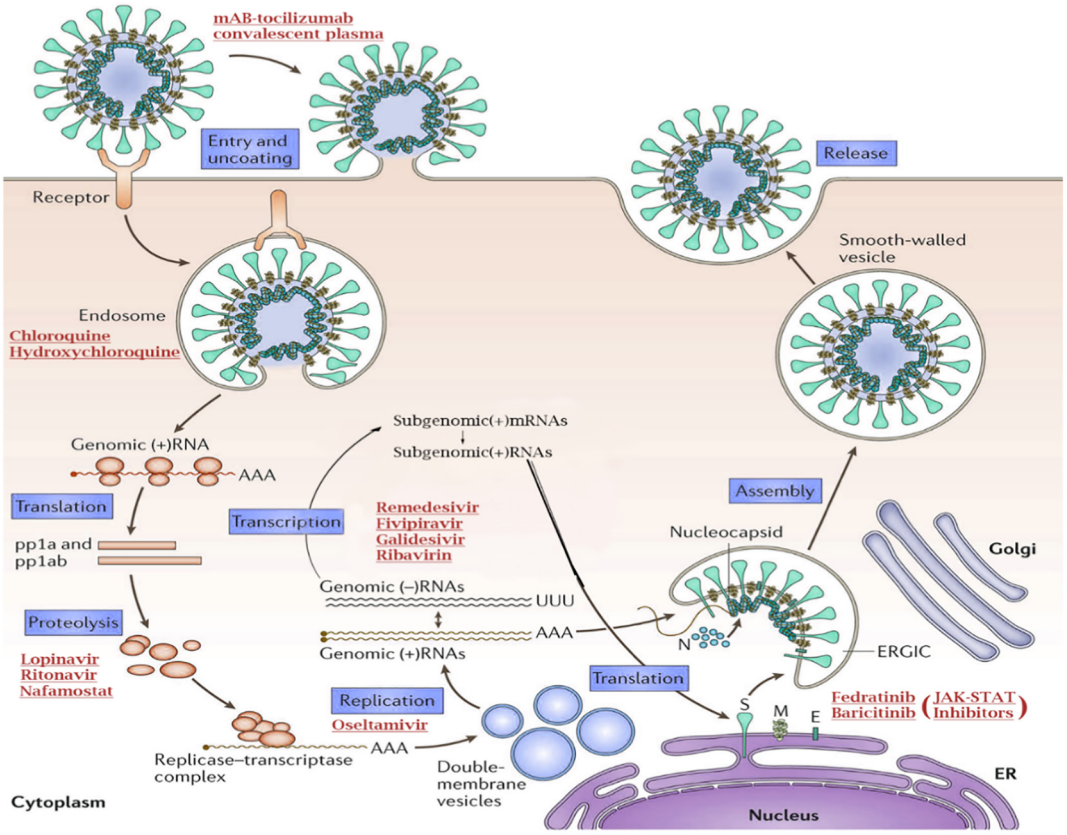

Fig. 8. The life cycle of SARS-CoV-2 showing potential drug targets in the host cell. The S protein of the virus binds to the cellular receptor (ACE2) followed by the entry of the viral RNA genome into the host cell. After the genome entry into the cell translation of structural and NSPs follows. ORF1a and ORF1ab are translated to produce polyproteins pp1a and pp1ab, which are further cleaved by the proteases that are encoded by ORF1a to yield 16 non-structural proteins (nsp1-nsp16). Assembly and budding into the lumen of the ERGIC (Endoplasmic Reticulum Golgi Intermediate Compartment) then follow. Virions are finally released from the infected cell through exocytosis. In this life cycle of coronavirus, multiple stages are being seen as potential druggable targets, and drugs working like S protein inhibitors, RNA dependent RNA polymerase inhibitors (remdesivir, favipiravir, galidesivir, ribavirin), protease inhibitors (lopinavir, ritonavir, nafamostat), drugs altering the endosomal pH (chloroquine, hydroxychloroquine), JAK-STAT inhibitors (fedratinib, baricitinib), monoclonal antibodies (tocilizumab) have been proposed to show promising effects against the novel virus. Taking cas- based approach from previously encountered viruses like SARS and MERS many drugs are facing clinical trials. This figure was adapted from reference [103].

causes an impaired release of the virus from the endosome or lysosome and thus recommended to handle severe COVID-19 patients [120]. Hydroxy-chloroquine, a less toxic derivative of chloroquine, also found is effective in inhibiting SARS-CoV-2 infection [121]. Anti-inflammatory drugs like ibuprofen or cortisone sometimes recommended controlling the infection [122].

To understand the SARS-CoV-2 infection at the molecular level, it is essential to know the renin-angiotensin-aldosterone system (RAAS) hormone system that is central to SARS-CoV-2 infection (Fig. 9). The angiotensinogen is converted into angiotensin I by plasma renin released by the liver which is subsequently converted to angiotensin II by the ACE found on the surface of vascular endothelial cells of lungs. Angiotensin II acts on the AT1 receptor to cause vasoconstriction and also stimulates the secretion of the hormone aldosterone, which increases the reabsorption of sodium, thereby increasing blood pressure. Angiotensin I and II are degraded by ACE2 to angiotensin (1–9) and angiotensin (1–7), respectively. These molecules act on the Mas receptor to effect vaso-dilation thereby counteracting the effects of angiotensin II. SARS-CoV-2 infection requires the binding of the virus to the membrane-bound form of ACE2 and internalization of the S protein of the virus to the extracellular domain of ACE2, a membrane receptor, with a high affinity of 15 nM to be internalized by the host cell [24,123].

In the last few months, the following hypothesis has emerged about COVID-19 infection and cardiovascular diseases. Whether or not RAS blockers would be beneficial to COVID-19 cases is still controversial. Many patients with hypertension or other cardiovascular diseases are routinely treated with RAAS blockers and statins. However, clinical concerns remain whether these patients are at greater risk for SARS-CoV-2 infection due to enhanced ACE2 expression [124]. However, this has been argued against Huang and his group [125] who have shown that ACE inhibitors (ACEIs) and angiotensin receptor blockers (ARBs) have few effects on increasing the clinical severe conditions of COVID-19. It would, therefore, be fair to conclude here that more laboratory and clinical evidence are required to establish the roles of anti-hypertensive agents, ACE2 expression outcomes of COVID-19 in patients with cardiovascular disease [126].

Many different approaches have been adopted to tide over the COVID-19 pandemic [127–132]. Few of therapeutics used and their lacunae have been enumerated: (i) chloroquine phosphate: acute poisoning and death [133]; (ii) Lopinavir/ritonavir combination: randomized control trial not conducted [134]; (iii) Ibuprofen: safety concerns [135]; (iv) hydroxyl-chloroquine: randomized control trial not conducted [62]; (v) umbilical cord mesenchymal stem cells: still under study [136], (vi) Tilorone: broad-spectrum anti-viral (not specific) [137], (vii) losartan (ACE2 receptor blocker): hypothetical proposition [138], and (viii) Intravenous immunoglobulin collected from recovered coronavirus patients: SARS-CoV-2 contamination [139], (ix) blood

Table 2
List of drugs that have been found to have clinical effectiveness in COVID-19 therapy.

S. No	Drug	Target	Impact	Reference
1.	Remdesivir	Abroad-spectrum antiviral inhibits RdRP of RNA viruses, including SARS-CoV and MERS-CoV.	Adenosine analog, which incorporates into the nascent viral RNA chains during synthesis and causes premature termination.	[104]
2.	Chloroquine	Anti-malarial drug. Works at entry and post-entry stages of viral infection.	Increases endosomal pH required for virus/cell fusion. Interferes with the glycosylation of cellular receptors of SARS-CoV2.	[105,106]
3.	Fedratinib	JAK2 and FMS-like tyrosine kinase 3	Inhibition of JAK2 inhibits phosphorylation of STAT 3 and 5, which prevents cell division and induces apoptosis.	[107]
4.	Lopinavir	Protease inhibitor have *in vitro* antiviral activity against SARS associated coronavirus	Inhibition of coronavirus main proteinase interferes in the processing of polypeptide translation products.	[106]
5.	Oseltamivir	Neuraminidase inhibitor	Inhibits the neuraminidase activity of the virus subsequently prevents viral replication.	[108,109]
6.	Fivipiravir	RNA-dependent RNA polymerase	A guanine analog inhibits the RdRP activity of several RNA viruses (influenza, Ebola, Yellow fever and Chikungunya)	[110]
7.	Ribavirin	Nucleoside inhibitor An approved of HCV and RSV patients with SARS and MERS.	A nucleoside inhibitor that interferes with viral RNA synthesis and mRNA capping.	[111,112]
8.	Galidesivir	RNA polymerase	Disrupts RNA polymerase activity causes premature termination of the elongating viral RNA strand	[113]
9.	Nafamostat	Serine proteases inhibitor	Prevents membrane fusion by reducing the release of cathepsin B.	[104]
10.	Lianhuaqingwen	Herbal medicine commonly used for the prevention and treatment of viral influenza in China.	Chinese patent herbal medicine composed of 13different herbs played significant roles in the treatment of COVID-19.	[91]
11.	Baricitinib	Janus kinase (JAK) inhibitor	May block viral entry by inhibiting adaptor-associated protein kinase 1 and cyclin G-associated kinase	[114]
12.	Tocilizumab(mAB)	IL-6 inhibitor	Inhibition of IL-6 may attenuate pulmonary inflammation by controlling cytokine storm.	[110]
13.	Anti TNF alpha agents	TNF alpha	TNF-α promotes the production of other chemokine and cytokines, controls endotoxin-induced septic shock	[114]

purification therapy in reducing cytokine storm as a late complication of the disease [140]. SARS-CoV-2 vaccine pipeline holds a lot of promises that include whole virus vaccines, recombinant protein subunit vaccines, and nucleic acid vaccines. All these vaccines are currently under evaluation [141].

Even as the pandemic is growing in proportion and affecting millions across the world, there have been certain misconceptions about drug therapies for certain co-morbid conditions in COVID-19 patients [142]. The most important one pertains to the notion that NSAIDs cause an aggravation of COVID-19 infection. But with a lack of robust evidence, COVID-19 patients have been advised against self-medication with NSAIDs [143]. Also, paracetamol, an NSAID, is the choice of drug for fever, NSAIDs generally have no specific role in suppressing SAS-CoV-2. In terms of targeting the human immune system, the innate immune system is mostly recommended because it controls the replication and infection of CoVs [144]. In this regard, blocking the interferon signalling is expected to enhance the immune response. Also, blocking the signal pathways of human cells involved in virus replication was shown to have an effective antiviral effect.

In the quest to find a cure for COVID-19, WHO has conceptualized "SOLIDARITY", an international clinical trial as a common global platform. The advantages of this trial are manifold: (i) reduces the time taken by 80%, as compared to other trials; (ii) helps facilitate the rapid worldwide comparison of unproven treatments; (iii) overcome the risk of multiple small trials not generating the strong evidence needed to determine the relative effectiveness of potential treatments, and (iv) it looks to involve developers and companies to collaborate on ensuring affordability and availability of the treatment options if they prove effective. Based on the evidence so far from laboratories, animal studies and preliminary clinical studies, the treatment options of Remdesivir, Lopinavir/Ritonavir, Lopinavir/Ritonavir, Lopinavir/Ritonavir with Interferon beta-1a, have now been initiated [113,127,145].

In addition to discussed therapies, mesenchymal stem cell (MSC) therapy is a promising option currently implicated in the treatment of COVID-19 [146,147]. The study conducted by Leng et al. [148] on patients with COVID-19, suggested that intravenous transplantation of MSCs into patients is effective in the treatment of COVID-19 with lesser side effects. In should be noted that MSCs show immunomodulatory function. The successful infusion of MSCs resulted in increased pulmonary function of the subjected patients to this therapy. On the other hand, convalescent plasma emerged as a potential therapy for severe COVID-19 patients [132]. The use of convalescent plasma as a treatment was recommended by WHO during the outbreak of the Ebola virus in 2014. In the strategy, the convalescent plasma is retrieved from the fully recovered patients of viral disease and is transfused in the infected person as a treatment strategy. During the time of the COVID-19 pandemic, the successful application of this therapy is effective in some of the patients [149].

Tocilizumab is a monoclonal antibody against the Interleukin-6. During recent months, as the severity of COVID-19 elevates globally, it has been used as an alternative treatment strategy for COVID-19 patients [129]. The rationale for using tocilizumab, an Interleukin-6 inhibitor, is that in most COVID-19 affected persons the activation of T lymphocytes and mononuclear macrophages occurs in large numbers resulting in the secretion of interleukin-6 [128]. Excessive presence of Interleukin-6 causes cytokine storm and other inflammatory responses in the lungs and other organs [150]. The tocilizumab administration is used to control the elevated levels of Interleukin-6. The studies suggest that successful application of tocilizumab treatment in COVID-19 patients shows improvement in the condition of the patients with an average of 15 days from the start of treatment and resulted in decreased mortality [151].

As far as the drug resistance in coronaviruses is concerned, there are very few available studies that deal with the subject with intricate depth [152]. However, the studies conducted so far suggest the role of various mutations in the target proteins of the coronaviruses associated

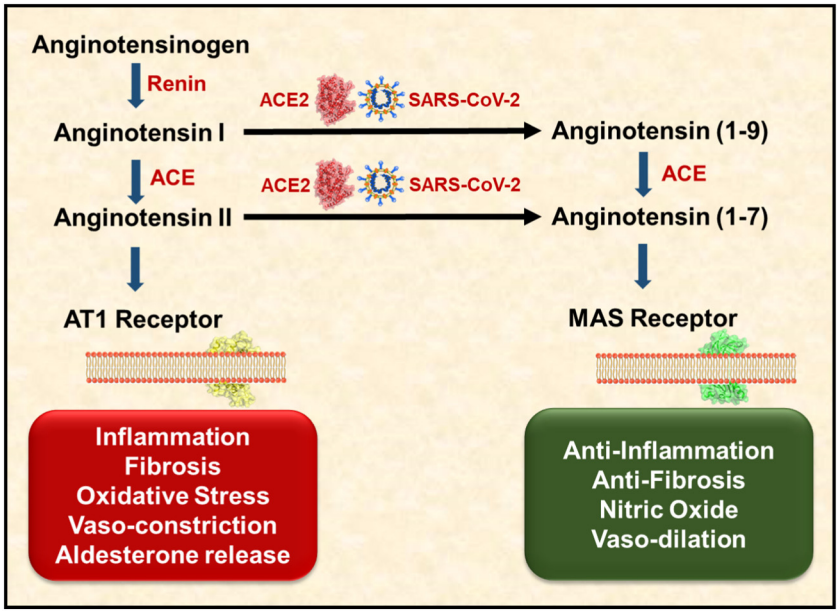

Fig. 9. Showing role of ACE2 in SARS-CoV-2 infection.

mutations to the drug resistance [153,154]. Still there is a need to conduct more studies focusing specifically on gene mutations that are responsible for drug resistance in coronavirus related maladies [155]. The best approach for the development of drugs for SARS-CoV-2 may be the use of available marketed drugs, validated through a well-defined pipeline of drug repurposing [156–158]. Recently, we have shown that FDA approved drugs, glecaprevir and maraviroc may be implicated as inhibitors of the main protease of SARS-CoV-2 to address COVID-19 therapy [159]. Once the efficacy of such drugs in the case of COVID-19 is determined, rapid clinical treatment of patients will be available.

9. Conclusion

The sudden emergence of pandemic SARS-CoV-2 has caused widespread fear and concern and has threatened global health security. The scientific community all over the globe is working rigorously to find an effective vaccine of drugs against the novel coronavirus. The genomic features of SARS-CoV-2 discussed in this study provide a possible hypothesis for the pathogenesis and transmission of the disease in humans. Efforts in the short term should be focused on developing a vaccine or inhibitors that help to prevent the infection by targeting the major viral proteins such as S, E, M, N, proteins, RdRP and proteases. The three infections have marginal differences in terms of innate immune responses but greatly differ in terms of morbidity and mortality. The detailed insights presented here might help to pave the way for understating how the novel coronavirus differs in its modus operandi compared to previously known strains. Our comparative study provides answers to some key questions relating to pathogenic mechanisms of SARS-CoV-2, in the context of developing potent drugs and vaccines against protein targets for the development of better approaches in COVID-19 therapy.

Declaration of competing interest

All authors of the manuscript declare to have no conflicts of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bbadis.2020.165878.

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