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Research Paper

Conserved Amino Acids in HCoV-HKU1 and SARS-Cov2 at RNA-dependent RNA Polymerase (RdRp) Motifs

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Abstract

A novel and related to severe acute respiratory syndrome associated coronavirus (SARS-CoV-2) has been identified as an infectious coronavirus originating from a Wuhan seafood market and rapidly spreading into and beyond China. While non-conserved regions of viral genome can lead to novel coronavirus subtypes, conserved regions are also very important for drug and vaccine researches. In our study, together with SARS-CoV showing high sequence similarity with SARS-Cov2, also genome and protein sequence alignments of non-lethal OC43 and HCoV-HKU1 viruses for SARS-CoV-2 were performed in the regions shown as drug targets. The genomic sequence similarities of SARS-CoV2 with SARS-CoV are 79% between 6-1923 bp, 82% between 3956-21577 bp, 80% between 22539-27910 bp and 90% between 28257-29894 bp, respectively. Major similar region of OC43 spike protein with SARS-CoV-2 is located in the C terminus, 2 other conserved regions with lower similarity were detected in N- terminus of S protein. HCoV-HKU1, has a 73% similarity between 14348-15992 bp in the genomic sequence of SARS-CoV-2 RNA-dependent RNA polymerase (RdRp). Similar regions of relatively low virulence HCoV-HKU1 with SARS-CoV-2 for RdRp can contribute understanding active sites of RdRp and developing a specific drug target. Antiviral approaches to the conserved regions of the RdRp protein have the potential to protect against existing and possible novel coronavirus species. Although lethal coronaviruses contain a large number of non-conserved regions compared to relatively low virulence respiratory pathogens, existence of the conserved regions for replication and understanding functional importance of these regions may be vital to develop effective treatment methods.

Keywords: COVID19; HCoV-HKU1; OC43; RdRp.

1. Introduction

A severe acute respiratory syndrome associated virus has been identified as an infectious COVID 19 (SARS-CoV2) originating from a Wuhan seafood market and recognized to be rapidly spreading into and beyond China [1]. Although various coronaviruses species were known for many years and we have encountered their deadly pathogenic species such as SARS-CoV and Middle East respiratory syndrome coronavirus MERS-CoV before, SARS-CoV-2 affecting countries all over the world has become primary focus of many researchers. Generally human Coronaviruses (HCoV) represent a wide range of positive RNA viruses that can cause respiratory, enteric, hepatic and neurological symptoms in human and animal hosts [2]. The first coronavirus species appearing earlier were thought to be only relatively low virulence respiratory pathogens. However, after emergencing of SARS-CoV and MERS-CoV, they have been accepted as one of the major pathogens in respiratory tract infection worldwide [3, 4, 5, 6].

Phylogenetic evidence shows that SARS-CoV and MERS-CoV are sourced from bats [4, 6, 7, 8] . A large number of alpha and beta coronaviruses have been discovered in bats. Phylogenetic evidence has shown that the last common ancestor of SARS-CoV is bat coronavirus RaTG13[9]. They are a natural reservoirs of alpha and beta coronaviruses, and will probably lead to increase novel coronaviruses due to their rich diversity and global distribution [4]. These observations suggest that novel coronaviruses can

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emerge soon besides SARS-CoV2. Various vaccines have started to be used from the end of 2020.

As the largest known RNA viruses, HCoVs are divided into four groups: alpha-, beta-, gamma-, and delta-coronavirus [10, 11]. HCoV-NL63 and HCoV-229E are included in alpha-CoV. On the other hand HCoV-OC43, HCoV-HKU1, SARS-CoV and MERS-CoV are members of beta-CoVs [5]. Also, SARS-CoV-2 is from the beta-CoVs.

The first human coronaviruses (HCoV-229E and HCoV-OC43) were identified in the 1960s [8]. It is known that the receptor for entry of HCoV-OC43 into a host cell is N-acetyl-9-O-acetylneuraminic acid [5, 12]. On the other hand, HCoV-229E binds to human aminopeptidase (hAPN) in a host cell [12]. APN is expressed on apical membranes of epithelial cells, synaptic junctions and antigen-presenting cells [14]. However, the receptor bound by HCoV-HKU1 on cellular surface is not known yet [5, 8].

HCoV-OC43 and CoV-HKU1 typically cause mild disease and are estimated to account for 15-30% of common colds [15]. Febrile seizures have been shown to be more common in children infected with HCoV-HKU1 than in children infected with HCoV-OC43 [16]. It was also reported that 2 patients, 66 and 74 years old with HCoV-HKU1 infection, with another serious underlying disease, have died [17]. Given presented cases infected with HCoV-HKU1 in various studies, it is thought that HCoV-HKU1 infection may require hospitalization in patients with another serious underlying disease and therefore [8, 16, 18, 19]. HCoV-HKU1 and HCoV-NL63 infections are generally accepted as not life threatening in healthy people [20].

Coronaviruses are positive-stranded RNA viruses with large viral genome (27 to 33 kb) of RNA viruses and share a generally protected organization [21, 22]. The coronavirus genome is translated into two protein groups in a host cell: Structural proteins such as Spike (S), Nucleocapsid (N), Matrix (M) and Envelope (E), and non-structural proteins such as RdRp (nsp12; Nuclear shuttle protein 12) and Helicase (nsp13) [8, 23, 24, 25]. There are 16 nsps playing a major role in RNA replication and transcription [25, 26]. After coronavirus enters a host cell, translation and replication of viral RNA occur in cytoplasm [23]. SARS-CoV-2 genome and annotations of its mature protein products were shown in Figure 1.

In the study, the conserved areas of relatively low virulence coronaviruses HCoV-HKU1 and HCoV-OC43 were analyzed and importance of their similarities with SARS-CoV-2 were discussed.

While the non-conserved regions of viral genome can lead to novel coronavirus subtypes, conserved regions are also very important for drug and vaccine researches. Could targeting the conserved regions generate a widely established immune response of the host cell against wide variety of different coronavirus subtypes? These type of questions remain to be explored and accordingly, we aimed to answer some of these in this study via bioinformatics based computational analyses.

2. Materials and Methods

2.1. Sequences

Nucleotide and amino acid sequences are retrieved from The National Center for Biotechnology Information (NCBI) GenBank and UniProt databases. Viral genome sequences are retrieved from Genbank (https://www.ncbi.nlm.nih.gov/genbank/).

The Genbank accession numbers are given in Table 1. The amino acid sequences of viral proteins are retrieved from UniProt (https://www.uniprot.org/). The UniProt accession numbers of sequences under investigation are shown in Table 2.

2.2. Genome annotation

Genomic region annotations are depicted via University of California Santa Cruz (UCSC) Genome Browser interface (https://genome.ucsc.edu/cgi-bin/hgTracks?hgsid=1117754485).

Genomic annotations are retrieved from USCS Genome Browser by using UniProt Protein Annotations.

2.3. Sequence alignments

The Basic Local Alignment Search Tool (BLAST) under NCBI website (https://blast.ncbi.nlm.nih.gov/Blast.cgi) is used to generate genome and protein sequence alignments.

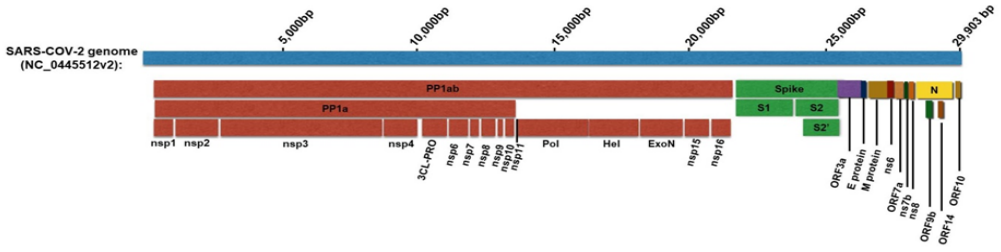

Figure 1. Depiction of SARS-CoV2 genome and annotations of its mature protein products. Complete genome of SARS-CoV-2 is shown in blue. Polyprotein 1 ab (PP1ab) is composed of 16 nonstructural proteins (nsp1-nsp16) and depicted in red. Four structural proteins; S, M, E and N are annotated in green, dark blue, brown and yellow, respectively. The 3' end of the viral genome contains eight nonstructural proteins (ORF3a-ORF10).

Multiple sequence alignments for amino acid residues indicating consensus above 70% are processed using Clustal Omega [28] and visualised using MView [29] under The European Bioinformatics Institute (EMBL-EBI) website: (https://www.ebi.ac.uk/Tools/msa/clustalo/).

Table 1. The GenBank accession numbers of viral genome sequences under investigation and their genome sizes are shown.

Virus	Accession number:	Genome Size (bp ss RNA)
SARS-CoV-2	NC_045512.2	29903
SARS-CoV	NC_004718	29751
HCoV-HKU1	NC_006577.2	29926

Table 2. The UniProt accession numbers of amino acid sequences under investigation and their aminoacid sizes are given.

Viral protein	Accession number	Size (aa)
SARS-CoV-2 Surface Glycoprotein	YP_009724390.1	1273
OC43 Spike Glycoprotein	P36334.1	1353
SARS-CoV-2 RNA-dependent RNA polymerase	YP_009725307.1	932
HCoV-HKU1 orf1ab polymerase	YP_173236.1	7182

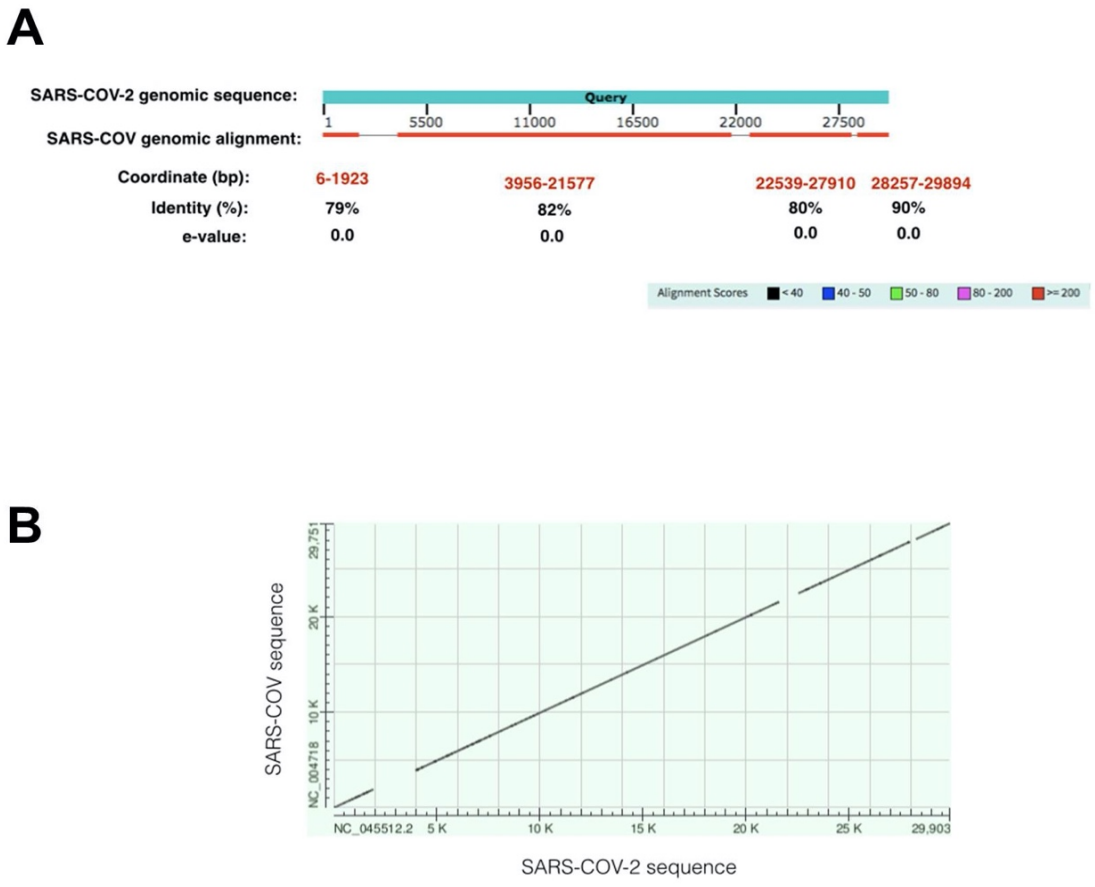

Figure 2. (A) Figure depicts nucleotide blast result between SARS-CoV-2 and SARS-CoV. Four regions are aligned to SARS-CoV-2 with depicted coordinates, percent identities and e-values. (B) Dot matrix shows regions of similarity based upon the BLAST results. SARS-CoV-2 sequence is represented on the X-axis and the numbers represent the bases. The SARS-CoV is represented on the Y-axis and the numbers represent the bases. Alignments are shown in the plot as lines.

3. Results

In our study, together with SARS-CoV showing high sequence similarity with SARS-CoV-2, also genome and protein sequence alignments of non-lethal OC43 and HCoV-HKU1 viruses for SARS-CoV-2 were performed in the regions thought as drug targets. As shown in Figures 2A and B, there is 82% similarity ratio between the entire genomes of SARS-CoV and SARS-CoV-2. However, A genomic sequence similarities of SARS-CoV2 with SARS-CoV are 79% between 6-1923 bp, 82% between 3956-21577 bp, 80% between 22539-27910 bp and 90% between 28257-29894 bp, respectively.

Morse et al. have shown that SARS-CoV-2 and SARS-CoV share 82% sequence identity at genomic RNA levels, but they share 96% sequence identity in active sites of RdRp proteins needed for the polymerization of RNA. Sequence variations were found to be located away from the active sites [1].

Also, HCoV-HKU1, has a 73% similarity between 14348-15992 bp in the genomic sequence of SARS-CoV2 RdRp (Figures 3). Similar regions of relatively low virulence HCoV-HKU1 with SARS-CoV2 for RdRp can contribute understanding active sites of RdRp and developing a specific drug target. Antiviral approaches to the conserved regions of the RdRp protein have the potential to protect against existing and

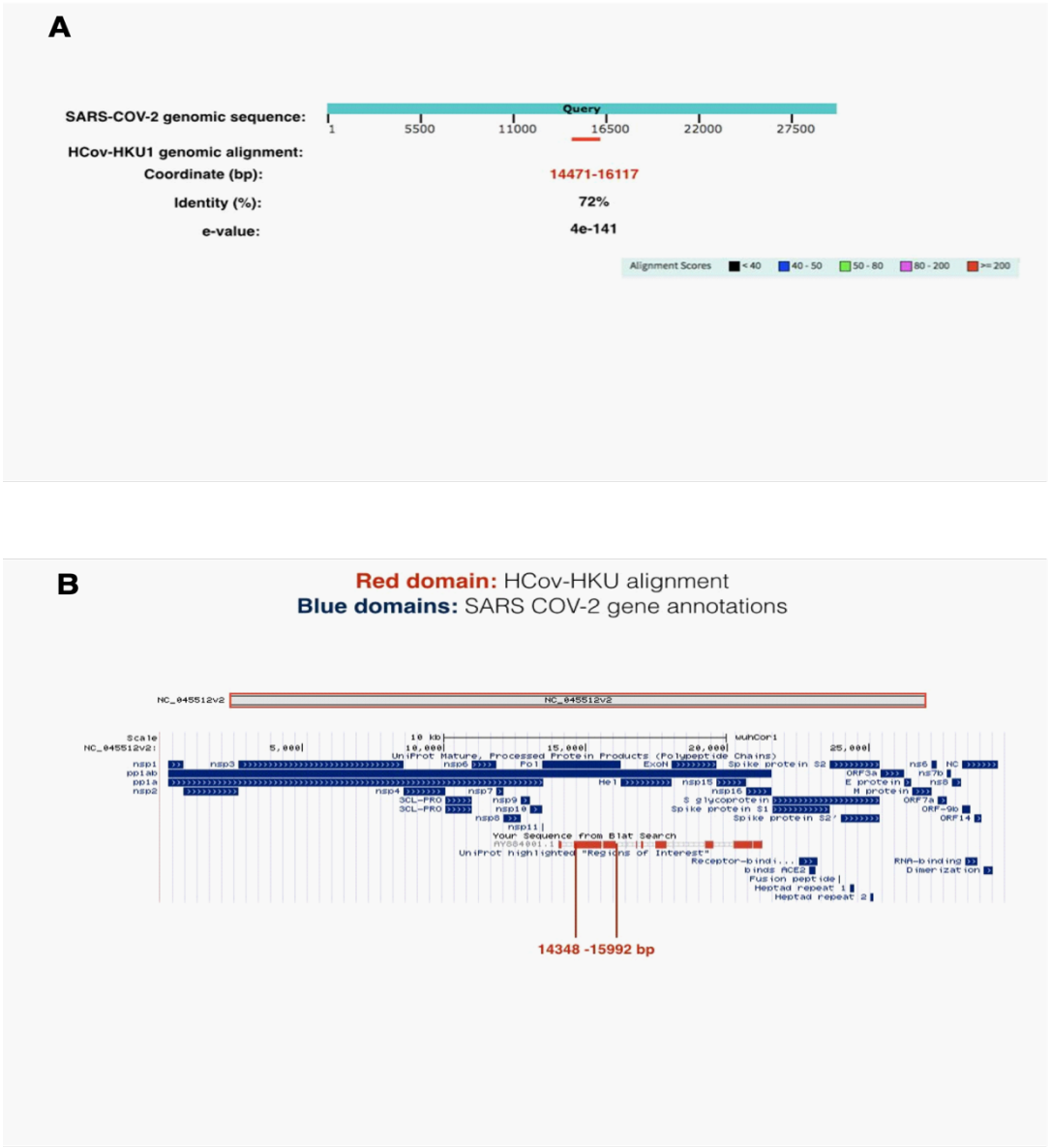

Figure 3. (A) Figure depicts nucleotide BLAST result between SARS-COV-2 and HCoV-HKU1. Red region is aligned to SARS-COV-2 with a percent identity of 73 with and e-value of 4e-146. (B) Genome browser screen depicts SARS-COV-2 vs HCoV- HKU1 alignment on Pol genomic region. Blue domains are UniProt protein annotations. Red region shows HCoV- HKU1 alignment. SARS-COV-2 coordinates between 14348-15992 bp are shown in red.

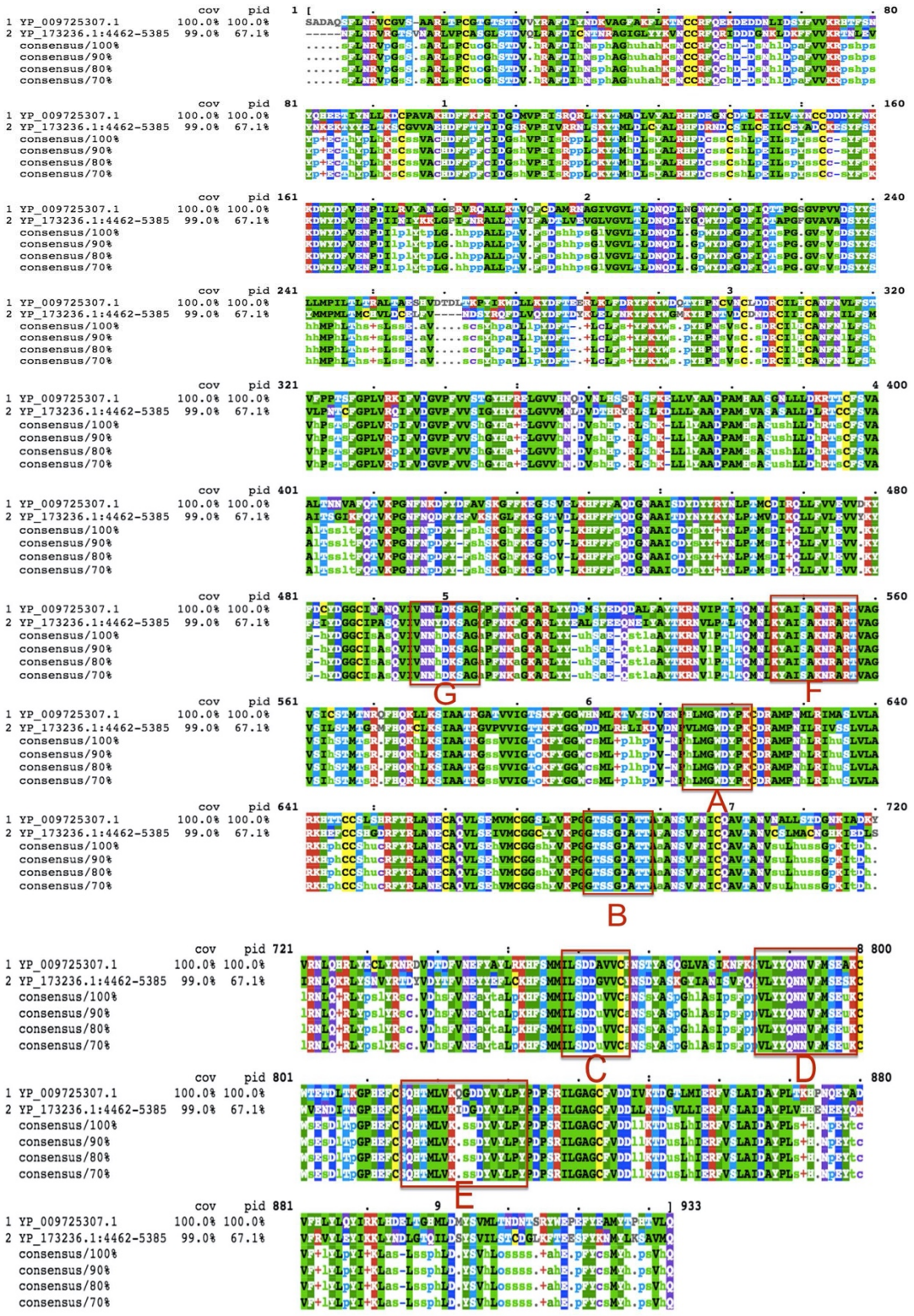

Figure 4. Amino acid sequence alignment of SARS-COV-2 RdRp (YP_009725307.1) and HCov-HKU1 Orf1ab (YP_173236.1: 4462-5385). Consensus indicates 70% and above. Cov is for 'coverage', pid is for 'percent identity'.The polymerase conserved motifs (A–G) stated as in Shannon et al. [27] are shown in red boxes. Alignments are processed and visualised using Clustal Omega [28] and MView [29].

possible novel coronavirus species. Although lethal coronaviruses contain a large number of non-conserved regions compared to relatively low virulence respiratory pathogens, existence of the conserved regions for replication and understanding functional importance of these regions may be vital to develop

effective treatment methods. It can also provide broader protection against these coronavirus species.

Amino acid sequence alignment of SARS-COV-2 RdRp (YP_009725307.1) and HCov-HKU1 Orf1ab (YP_173236.1: 4462-5385) were made (Figure 4) The polymerase conserved key functional motifs (A–G) stated as in Shannon et al. are shown in red boxes [27]. Similarity of amino acid sequences of HCov-HKU1 and SARS-COV-2 RdRp in the motifs the regions marked as B and F motifs are the same for the 2 viruses. There is only 1 amino acid difference in A, C, D and G motifs between the two viruses. There are 3 amino acid differences in E motif.

S proteins of coronaviruses are large transmembrane glycoproteins that mediate membrane fusion and viral entry [30, 31]. S proteins of some coronaviruses are divided into two subunits by cellular proteases. Two functionally different domains corresponding to the subunits of the split S proteins (S1 and S2) have been identified [32]. The S1 region mediates binding to a host cell surface receptor, while the S2 region contains hydrophobic fusion peptides and helical coil regions that regulate membrane fusion processes following receptor binding [15]. Residual amino acids 318 to 510 of the S protein bind to angiotensin converting enzyme 2 (ACE2) [33].

The similarity ratios of OC43 spike protein and SARS-CoV2 spike protein genome are shown in Figure 5. Between these two proteins, 3 regions have close similarities. The region marked in red is the most aligned region and has an amino acid similarity of around 38%. Regions shown in black and pink show 30% and 32% similarity, respectively. There is not much space between these similar sequences. Due to the amino acid differences caused by mutations, positively charged amino acid ratios were also examined. Accordingly, while the positively charged amino acid similarity was 54% in the red region with the highest similarity, 47% positively charged amino acid similarity was found in the region shown in black and 46% in the pink region. While N- terminus of spike protein is in extracellular area, C- terminus is in the intraviral area. Although a highly similar region of OC43 spike protein with SARS-Cov2 is located in the C terrminus, 2 other conserved regions with lower similarity were detected in N- terminus of S protein. However, according to the e value, there is no significant similarity at N terminus of OC43. Since the N terminus which resides in the extracellular region is not conserved, binding mode of S protein might be different between the two viruses.

The mutation rate of RNA viruses is one million times higher than their host cell, and this high rate is associated with virulence modulation, and evolvability for viral adaptation [34]. Could the presence of conserved regions of SARS-CoV-2 with non-lethal species in the same species, despite the high mutation rate of RNA viruses, mean that these regions are vital for the virus?

Biological characterization of viral mutations can provide a valuable information to evaluate viral drug resistance, immune escape and pathogenesis-related mechanisms. While mutations required for adaptation of viruses are inevitable for their survival, preference for their conserved regions for new vaccines, antiviral drugs, and diagnostic experiments may lead them to show efficient therapy in a longer time and wider spectrum.

3. Conclusion

Overall, genomic alterations do happen in coronaviruses leading to modifications in the genome. This in result may or may not return changes in the amino acid sequence at the protein level. Nevertheless, some of the critical regions in the genome remains highly conserved, since these regions remain as vital for the viral survival and accordingly these regions are selected during bottlenecks. Therefore, these facts bring three clear outcomes. First, the less lethal subtypes of coronavirus may be used model organisms of drug design approaches towards SARS-CoV-2. Second, identifying conserved regions in different coronaviruses may give clues for prediction of regions with high mutations rate, leading to the understanding why some coronavirus sub-types may results in more deadly disease conditions. Third, understanding converved and non-conserved regions and their reflection on three dimensional structures will lead to more comprehensive exploration of the functional outcomes of the mutations found in coronavirus subtypes. Thus, we believe the findings of this study is crucial to the understanding of the SARS-CoV-2 and suggests that in future chemical modulators targeting

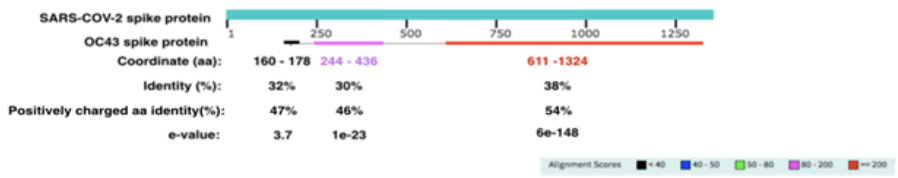

Figure 5. Figure depicts three protein alignment sites between OC43 and SARS-COV-2 surface glycoproteins. Amino acid residue coordinates, percent identities and e-values are as depicted.

conserved region of coronavirues carry the potential of identifying novel drug candidates against currently unidentified subtypes of this virus family.

RdRps is considered among the primary targets for antiviral drug development against a wide range of viruses. Some RdRp inhibitors such as Favipiravir, Galidesivir, Remdesivir and Ribavirin are thought to target SARS-CoV-2. Other possible drugs like Filibuvir, Cepharantin, Simeprevir and Tegobuvir are also estimated to be potential inhibitors of SARS-CoV-2 RdRp. Comparative research on activity of these drugs in conserved regions between the viruses can contribute to understanding the RdRp mechanism.

Conflict of Interest

The authors declare no conflict of interest.

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