The Phylogenetic Analysis of 5 SARS-CoV-2 Proteins' Sequences in Relation to Time and Geographical Location

Sophia Ying Li[1,†], Xiayan Li[2,*], Jiahe Gao[3], Kaiyang Pang[4] and Deyuan Xu[5]
[1]*Thomas Jefferson High School for Science and Technology, 6560 Braddock Rd Alexandria, VA 22312, U.S.A.*
[2]*School of Computer science and Engineering, Baylor University, 1311 S 5th St, Waco, TX 76706, U.S.A.*
[3]*Qingdao No.2 High School, Qingdao, Shandong, 266061, China*
[4]*Revelle College, University of California San Diego, 9500 Gilman Drive, La Jolla, 92093, U.S.A.*
[5]*College of Bioinformatics Science and Technology, Harbin Medical University, Harbin, Heilongjiang, 150076, China*

Keywords: Bioinformatics, Protein Sequence, SARS-Cov-2, Geographical Location, Phylogenetic Analysis, Evolution.

Abstract: After 18 months since the start of the COVID-19 epidemic, the virus continues to plague the world. Learning more about how SARS-CoV-2 proteins mutate and the relation to spatial location is critical in helping us predict the spread of variants. We built an MSA and UPGMA phylogenetic tree for each of the 5 crucial ORF sequences (S, M, E, N, and ORF1ab protein), which were collected by the latest update date for each region, and based on the results, we are not able to conclude that SARS-CoV-2 protein sequences from different countries co-evolved with other SARS-CoV-2 protein variants in proximity. However, some highly mutated regions within those sequences may suggest some evolutionary pattern during this continuing pandemic.

1 INTRODUCTION

1.1 Spread of Coronavirus

The outbreak of severe acute respiratory syndrome coronavirus (SARS-CoV-2) across the globe has had devastating impacts on various countries. In December 2019, the first COVID-19 infection, the disease caused by SARS-CoV2, was reported in Wuhan, China (Wang, Horby, Hayden, Gao 2020). The number of infections reports then increased rapidly around the world. In March 2020, COVID-19 was declared as a global pandemic by The World Health Organization (WHO) ("WHO Director-General's opening remarks at the media briefing on COVID-19 - 11 March 2020) As of September 2021, the CDC has reported almost 40 million cases and over 600 thousand deaths in the United States of America (CDC 2021). There has been a total of around 218.9 million cases and 4.5 million deaths (WHO Coronavirus (COVID-19) Dashboard). Although the epidemic is currently under control, COVID-19 is still spreading in some countries and areas, threatening global health systems and health security. For the foreseeable future, novel coronavirus outbreaks will continue for several years, and national and regional prevention measures will continue. Novel coronavirus, a part of the Coronaviridae family, causes severe respiratory infections in mammals. According to the World Health Organization, based on the accumulated observations, the most common symptoms of COVID-19 are fever, dry cough, and fatigue. Less common symptoms include loss of taste or smell, nasal congestion, conjunctivitis (also known as red eyes),sore throat, headache, muscle or joint pain, and nausea (Coronavirus disease (COVID-19).''). There have also been many asymptomatic cases, as seen through a study of healthcare workers by Wilder-Smith et al (A. Wilder-Smith, M. D. Teleman, B. H. Heng, A. Earnest, A. E. Ling, and Y. S. Leo 2005).

1.2 Geographical Location and Mutations of SARS-Cov2

One study by Fan et al. predicted the outbreak to come from bats and China because of the data from past SARS-related coronavirus outbreaks. The study predicts hotspots for the emergenceof the virus using 3 factors: recombination from rich gene pools, the distance between bats and humans, and virus transmissibility. The researchers found the spread of many diverse and closelyrelated CoVs between bats

Li, S., Li, X., Gao, J., Pang, K. and Xu, D.
The Phylogenetic Analysis of 5 SARS-CoV-2 Proteins' Sequences in Relation to Time and Geographical Location.
DOI: 10.5220/0011381500003443
In *Proceedings of the 4th International Conference on Biomedical Engineering and Bioinformatics (ICBEB 2022)*, pages 1164-1173
ISBN: 978-989-758-595-1

of various provinces in China (Fan, Zhao, Shi, Zhou 2019). Their result indicated a positive relationship between short spatial distances and bat coronavirus mutation rate.

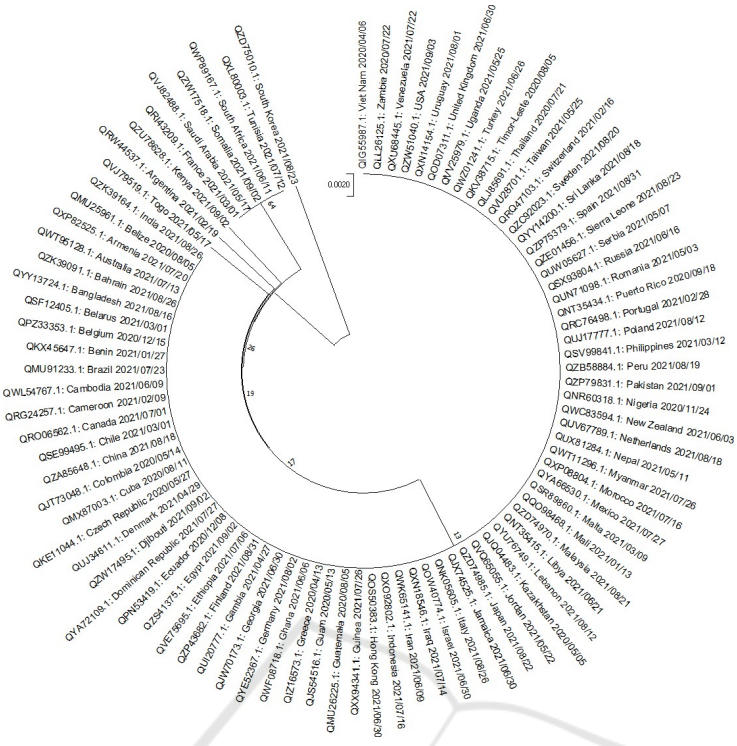
1.3 Coronavirus Proteins

Novel coronavirus is a single-stranded RNA virus that consists of 4 structural proteins: surfaceor spike glycoprotein (S), membrane glycoprotein (M), envelope protein (E), and nucleocapsid phosphoprotein (N). In the SARS-CoV2 genome, there are 10 operating reading frames (ORF), and the first ORF (ORF1ab) encodes for 1-16 non-structural proteins and phosphoprotein a and b,representing the biggest gene in the coronavirus's genome (Satarker, Nampoothiri 2020). The unique sequences which ORF1abcontains have been recognized as potential early detection targets for novel Coronavirus (Corman et al 2020, Jung et al 2020, Wang et al 2020). Therest code for the structural and accessory proteins. The M protein is present in high amounts and helps mediate the inflammatory response in hosts. The E protein is a tiny integral membrane protein that enhances viral pathogenicity and aids in virion assembly by producing viroporins. The Nprotein enhances viral entry and plays a critical part in virus transcription and assembly (McBride, M. van Zyl, Fielding 2014). The S protein is also known as surface glycoprotein or spike protein. It plays an important role in conformational rearrangement to membrane fusion, which creates pores on the host transmembrane that viral genomes can be passed through during the viral transmission. Specifically, the peptide 353- KGDFR-357 (H. sapiens ACE2 residue numbering), located on the surface of the ACE2 molecule,participates in the binding of the SARS-CoV-2 receptor-binding domain (RBD) (Huang, Yang, Xu, Xu, Liu 2020). The ACE2 receptor is expressed in lung, intestine, kidney, and epithelial alveolar type II cells.[13] Therefore, the study of mutation patterns within S protein may be crucial in understanding virus reproductiveadaptability and evolutionary survival in fast. These proteins are the building blocks of the virus, and understanding their sequence is crucial in understanding their function. However, due to the high mutation capacity of SARS-CoV-2 and its increasing adaptability to the environment, more research is still needed to eliminate the effects of the virus and restore the normal functioning of society. In particular, the Delta variant has been spreading faster than other variants due to its reduced sensitivity to antibodies that target the S proteins (Planas et al 2021). This study focuses on understanding the evolutionary pattern among the ORF1ab, S, M, E, and N of SARS-Cov2 from different countries. By comparing multiple sequence alignment (MSA) and analyzing phylogenetic trees, this study aims to discover the correlation between mutation rates and geographical location.Specifically, this includes the results suggesting whether specific protein sequences are conservedor highly mutated depending on the region of origin.

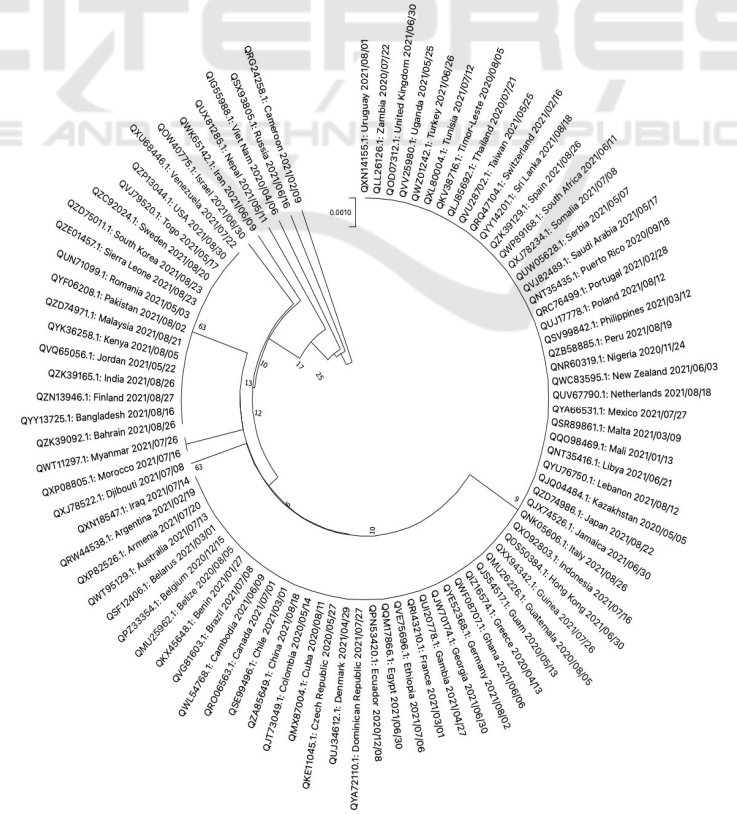
2 RESULTS

2.1 Minimal Relationship between Mutation of Different Proteins and Geographical Location

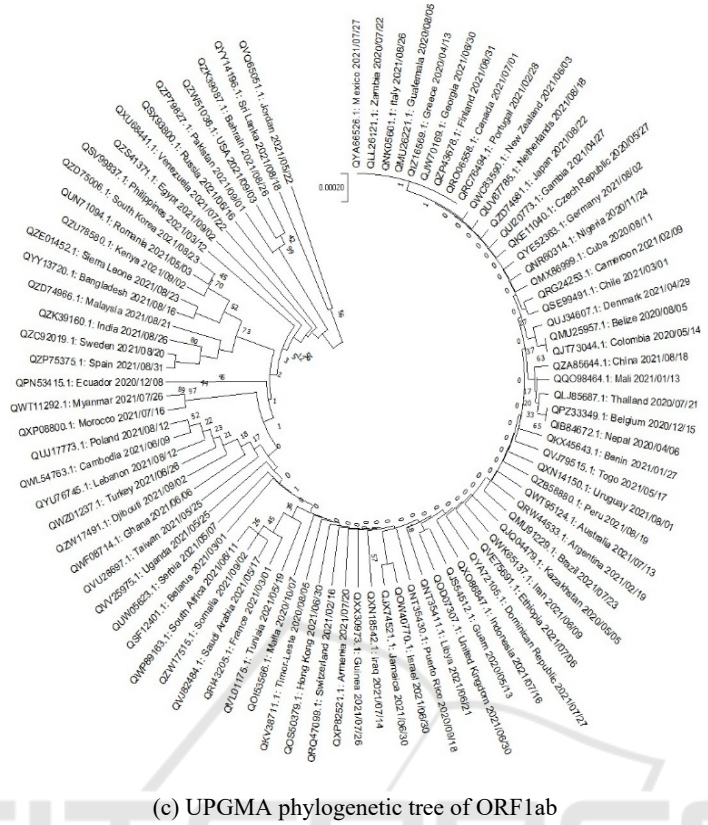
Based on the 5 protein UPGMA phylogenetic trees (see Figure 1-2), there is minimal correlationbetween specific protein mutation rates and geographical location. Moreover, there seems to be norelationship between S, M, E, Orf1ab, and N. Each tree has a significantly different structure based on which region each protein sequence came from. This means these proteins do not evolve with one another.



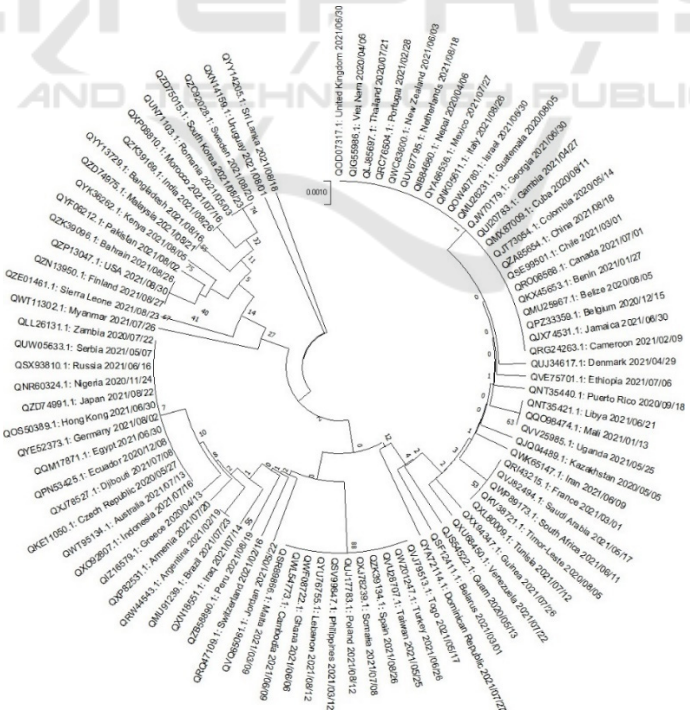
(a) UPGMA phylogenetic tree of envelope protein (E)



(b) UPGMA phylogenetic tree of membrane glycoprotein (M)



(c) UPGMA phylogenetic tree of ORF1ab



(d) UPGMA phylogenetic tree of surface Nucleocapsid protein (N)

Figure 1: UPGMA phylogenetic tree of relatively conserved SARS-CoV-2 proteins' sequences from various countries.

2.2 MSAs Indicate There to Be No Specific Mutation Point

There are no major similarities between where mutations of amino acids occur in any MSAs of each protein [see Figure 3-6]. The difference between each region's amino acid sequences is relatively random indicating there are no mutation points within any of the 5 proteins. However, this does not mean that there are no differences between the sequences. Each protein has variations between sequences from different countries, and S protein evolves and mutates most of the 5 proteins analyzed.

2.3 M, ORF1ab, N, and E Protein Are Highly Conserved between SARS-Cov-2 of DifferentCountries

The phylogenetic tree of M protein has few branches, and there are only a few differences between the M protein sequences of the 89 countries. The one major variation is at position 82aa of 222 aa where 16 sequences have threonine, 70 have isoleucine, and 2 have serine. Serine and threonine are polar amino acids, while isoleucine is nonpolar. This may affect the structure of the protein. 3D structure prediction is needed to understand the impact of this variation. Unlike the other countries' sequences, the sequence from Iraq and Cameroon has a long section of 'undetermined or atypical amino acids' near the beginning and the end respectively. Overall, the phylogenetic tree and MSA suggest that the M protein sequence is highly conserved. For ORF1ab,there is one highly mutated region observed from the MSA at 3667 aa - 3691 aa, and there is a gap introduced in Lebanon, Philippines, Venezuela, Ghana, Taiwan, Djibouti, Cambodia, Poland, Dominican Republic, Tunisia, Saudi Arabia, South Africa, France, Togo. However, this region isnot fully studied, so it is hard to draw any conclusion on the contribution of this region to the evolutionary pattern. Sequences of N protein were analyzed, and the mutation rate is relatively high at 503 aa. In most protein sequences, the amino acid at this position would be asparagine. However, there are 15 sequences where the amino acid of this position is tyrosine. By extracting the table corresponding to the countries of those 15 sequences, it turns out that 13 of those sequences come from mid-latitude regions if not low-latitude regions. These countries are mainly under the influence of tropical climate or subtropical climates. According to the MSA analysis, sequences of E protein in different regions are comparatively similar. Moreover, because there are also onlya few branches in the phylogenetic tree, E protein is likely to have a highly conserved sequence between different countries. The most noticeable differences are ones in a few tropical countries:Somalia, Saudi Arabia, and Kenya. They follow the highly conserved consistency and mutate only in particular regions. The biggest difference is a mismatch at 71 aa, in which 5 countries have leucine and the rest have proline.

2.4 S Protein Is Highly Mutated between SARS-Cov-2 of Different Countries

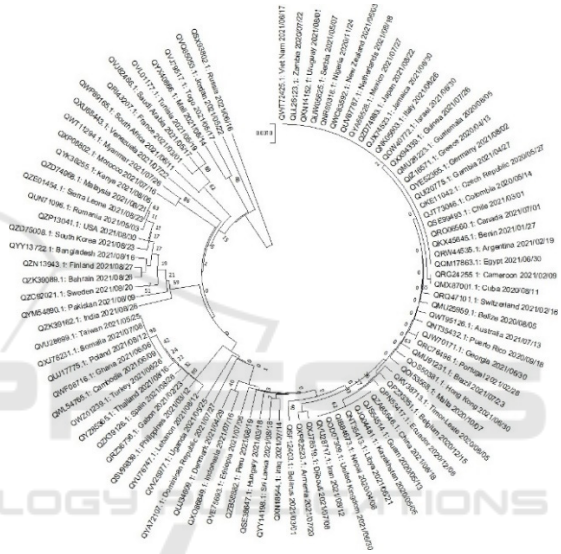


Figure 2: UPGMA phylogenetic tree of highly mutated SARS-CoV-2 of surface glycoprotein(S).

There are numerous gaps and mismatches detected from the MSA outcome of S protein: gaps aredetected from 57 aa - 263 aa and 682 aa - 686 aa (this gap is mainly caused by the 4 aa insertions from the Russia sequence), and few mismatches are detected at the regions close to the gaps. This may suggest these regions undergo positive evolution. Within the UPGMA Phylogenetic tree graph, the distance unit, which is 0.001, for this tree is relatively larger than the M, N, E, and ORF1ab protein. Surprisingly, the sequence from Russia has a long-stretched branch at the beginning of the root tree, which may be caused by 4 aa insertions from 682 aa - 686 aa, and the other region sequences which have introduced gaps, also are at the position close to the root. Thereare also no obvious spatial or time relations captured within the protein S phylogenetic tree.

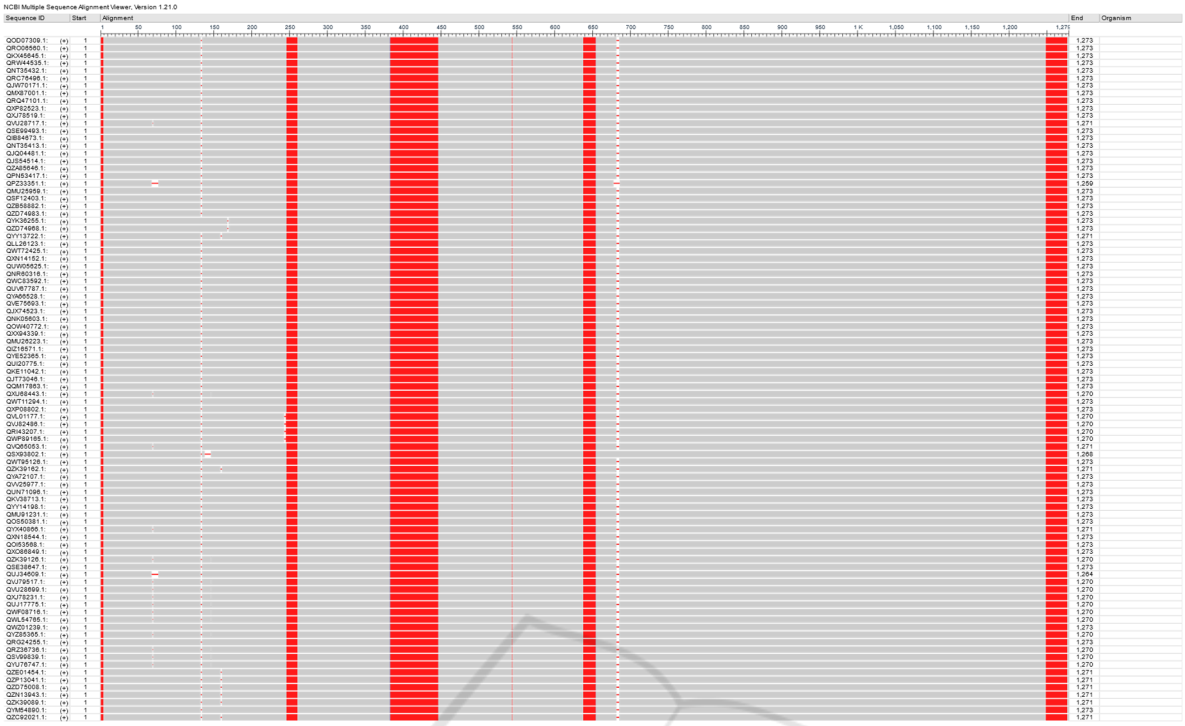


Figure 3: Protein S frequency-based difference MSA.

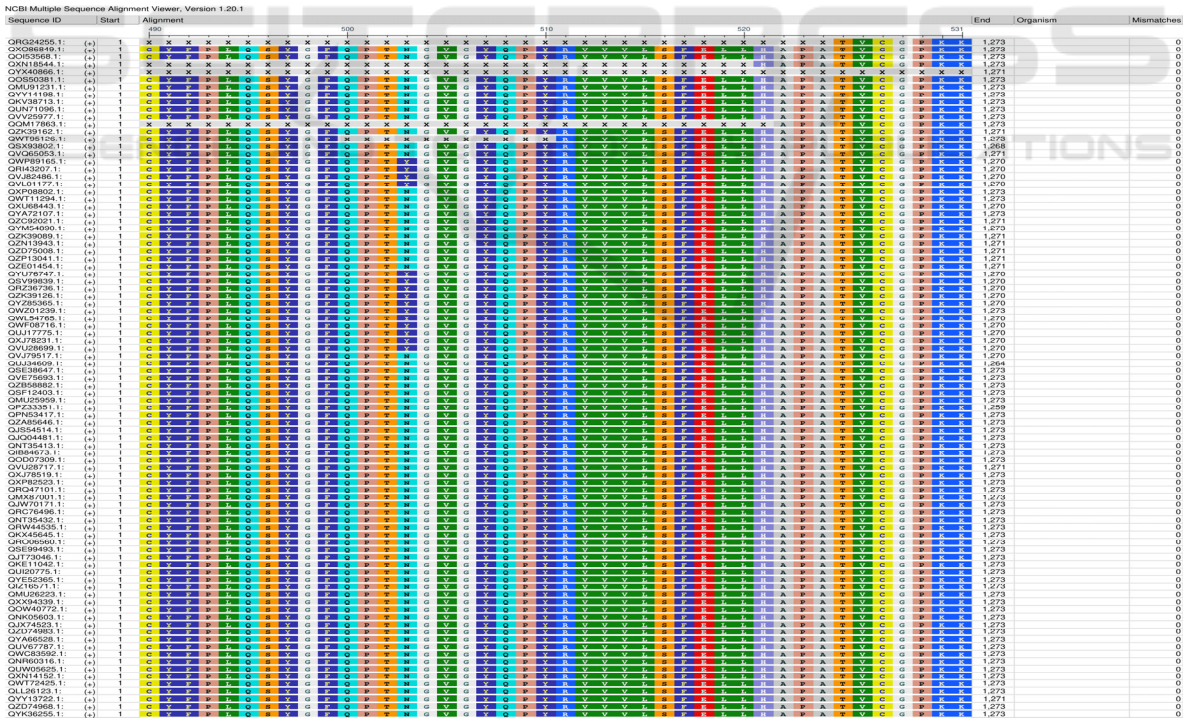


Figure 4: MSA snapshot of protein N.

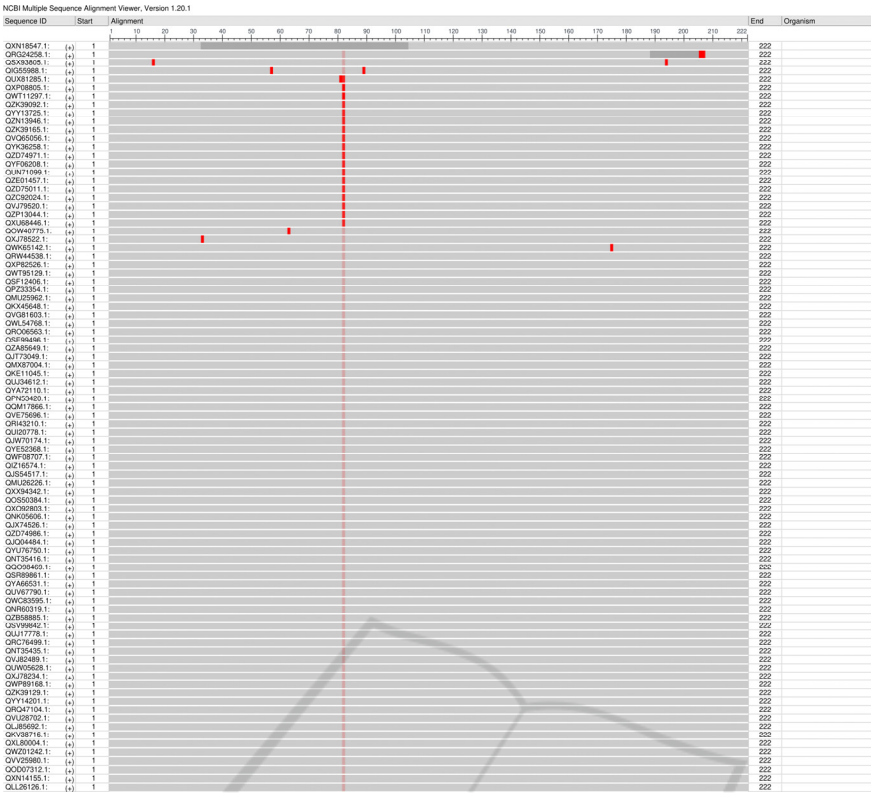


Figure 5: Protein M frequency-based difference MSA.

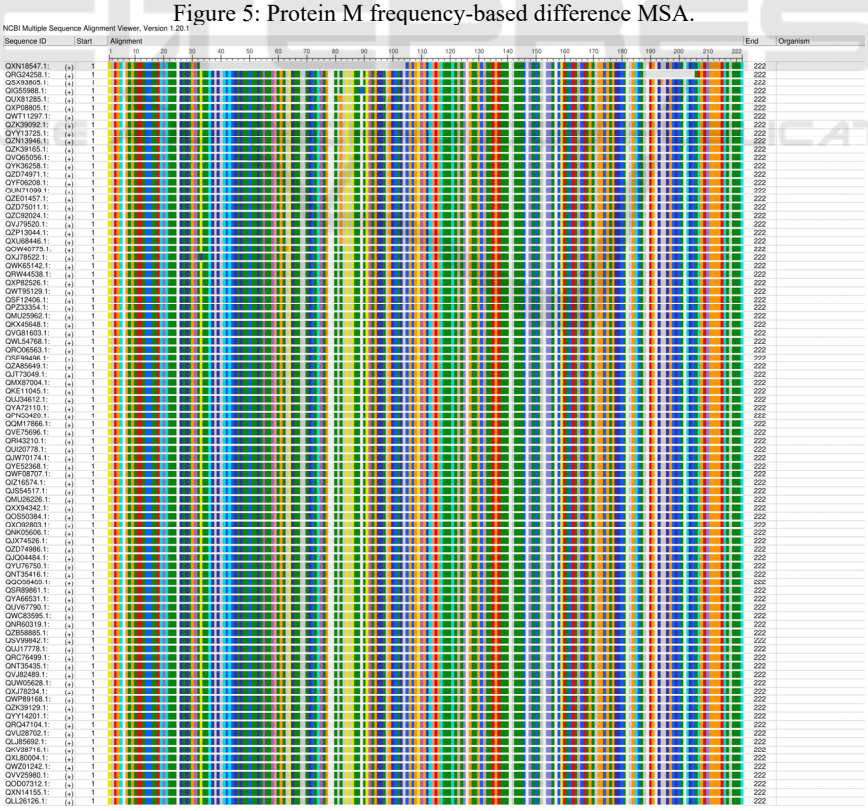


Figure 6: Protein M rasmol amino acid coloring.

3 DISCUSSION

3.1 Geography and Time Have an Insignificant Role in SARS-Cov-2 Evolution Pattern

In the UPGMA phylogenetic tree of the 5 proteins, it is hard to conclude that location and time play a role in the SARS-CoV-2 evolutionary path. For instance, the latest sequences for countriesin North America on the S protein phylogenetic tree is scattered: USA is on branch 17, but Canadais on branch 0. Moreover, the protein sequence QNT 35432.1, Puerto Rico, 2020/09/18 updated in 2020 is not close to the root, which may be due to global traveling. Traveling makes the contact of individuals from non-neighboring regions easier, so the study of sequences from a region with lesstraveling may help eliminate this factor. Moreover, this introduction of a new variant may be the reason that unrelated protein sequences are similar. Further studies are needed to understand the mechanisms related to the mutation rates of SARS-CoV-2 proteins. A similar study on SARS-CoV SNVs (Single nucleotides variations) also suggests that SNVs frequency across different regions may be different by geography and time (Chen, Altschuler, Zhan, Chan, Deverman 2021). Therefore, there are minimal effects from geography and time on the small-scale (nucleotide) evolutionary pattern, and this result may be similar to theminimal effects of geography and time on a larger scale (sequences).

3.2 Difference between Highly Mutated and Conserved Proteins

The phylogenetic tree of the ORF1ab protein has a smaller scale compared to the phylogenetic tree of other proteins. Because this protein consists of 2/3 of the RNA and encodes for the nonstructuralproteins, the results strongly suggest the sequence of this protein is highly conserved between SARS-CoV-2 of different countries. Considering the function of N and E protein have with the virus assembly, the process is highly vulnerable to the temperature of the environment. In other words, the difference occurring at 503 aa of N protein and 71 aa of E protein may be the adaptivityof the proteins to the climate, further altering the virus' survival and spreading under different climates. More research on the temperature sensitivity of N protein is needed to understand this difference. The highly conserved nature of M proteins is likely related to its function in helping the virus survive in host cells as it inhibits NFB (Nuclear Factor Kappa B), which needs to be activated to produce an immune response to pathogens. This process is specific because of the direct interaction between M protein and I$\kappa\kappa\beta$ (I Kappa B Kinase) (Fang et al 2007). Noticeably, Protein S MSA outcome reveals two highly mutated regions occurred from 57 aa - 263 aa and 682 aa - 686 aa in sequence, and those highly mutated regions are found in the N-terminal domain (NTD) of the S1 subunit and S1/S2 cleavage regions. The second highly mutated regions are 6 - 10 aa upstream ofthe cleavage site, which this site should be cleaved during virus egress, and highly mutation patterncaptured within this site may suggest that the variability of this region provide a gain-of-function tothe SARS-CoV-2 for efficient spreading in the human population compared to other lineage b beta coronaviruses (Coutard 2020). Since the comparison made is between countries, it may also suggest that this virus is highly adaptable within a changing environment. Additionally, evidence shows that NTDof the S1 subunit is involved in promoting cytokine release in immune cells, and this appearance ofcytokines can lead to respiratory failure and a fatal outcome (Chan et al 2021). Thus, the frequent mutation within NTD-S1 subunit regions may give rise to varying severity of immune response across the differentmutant strains and adaptive attack pathways towards different races of peoples' immune systems. In general, the mutation pattern of SARS-CoV-2 Protein S may assist the rapid spreading rate andacute immune response during the evolution process.

3.3 Future Direction

Combining the study of human immune response variants towards SARS-CoV-2 may be consequential for understanding how the mutations get selected in the co-evolution with the host immune system mechanisms, and the patient's record from the GWAS catalog can be a valuable resource for analysis. In addition, understanding how these mutations affect each of the protein structures will be crucial in finding further therapeutic options and improving vaccine booster shots.

4 METHODS

4.1 Protein Sequences Collection

The latest sequences of ORF1ab polyprotein, spike glycoprotein, envelope protein, membrane glycoprotein, nucleocapsid phosphoprotein were

collected for each region ("INSDC Country List.) if the data was present. In this study, these sequences were collected by a script, which is available at github repository. Entrez-direct (Kans 2021) an Unix command-line tool that provides access to the NCBI interconnected database, was used to collect the protein ID, regions name and update dates, and download sequences in FASTA files format. There were 88 sequences collected for ORF1ab, 91 sequences collected for protein S, 89 sequences collected for Protein E, 89 sequences collected for Protein M, and 89 sequences collected for Protein N. The header for each sequence in the FASTA file was substituted into the format of "protein id:region name updateDate" to produce straightforward visualization for further analysis, and "fixed sequences.fasta" file stores the fixed header and aminoacids sequence. All sequences for five proteins were collected on 09/04/2021, and the MSA and Phylogenetics Tree analyses were made on the same day.

4.2 MSA Analysis

The five sequences FASTA files were separately aligned by MUSCLE, a tool for creating multi- ple alignments of protein sequences in high biological accuracy and time efficiency (Edgar 2004). No specialparameters were set except the input file and output file because the alignments were relatively short and there were few alignments. After running MUSCLE on a five sequence FASTA file, it produced the alignments files which insert gaps to achieve the maximum sum-of-pairs (SP) score.The 5 alignment files were the input of the MEGA X tool to make the UPGMA phylogenetic trees.Because it was hard to capture the mismatch and gaps in 5 alignment files, NCBI Multiple Se- quence Alignment Viewer (NCBI Multiple Sequence Alignment Viewer) a graphical display for multiple alignments of nucleotide and proteinsequences, is used to produce a better graphic visualization of MSA analysis results. The partial or whole scope of MSA outcome was captured to show the significant regions.

4.3 UPGMA Phylogenetics Tree Analysis

Finally, the UPGMA phylogenetic trees were created by MEGA X (Kumar, Stecher, Li, Knyaz, Tamura 2018) a software that implements tools for phylogenomic analysis. 'Bootstrap method' -> 'Test of Phylogeny', '500' -> 'No. of Bootstrap Replication', 'Amino Acid' -> 'Substitutions Type', Poisson model' -> 'Model/Method','Uniform Rates' -> 'Rates among sites', 'same (Homogeneous)' -> 'Pattern among Lineages', and 'Pairwise deletion'->'Gaps/Missing Data Treatment' were set for the progress, and rooted trees were kept in a circle format.

5 CONCLUSION

By analyzing the UPGMA phylogenetic tree and MSAs of each protein, the results show that geographical location has an insignificant impact on SARS-CoV-2 protein mutations and relationships.A few potential highly mutated regions among the sequence of each region may suggest the dynamic adaptivity to diverse environments and alternative invading strategies to human immune response. Because geographical location and time do not have a direct relationship to SARS-CoV-2 protein mutations, there may be a more complex underlying factor to explain the relationships between the protein sequences of various SARS-CoV-2 which can be studied in the future. Carriers such as different animals and humans may be one of the many factors that contribute to this similarity between protein sequences. Variability in the human immune system may also be a fac-tor that causes indirect relations between temporal, geographical location, and SARS-CoV-2-point mutation patterns. Therefore, more studies about how the human immune system evolved during the pandemic should be analyzed. This could be done by researching patients' variants record from the GWAS catalog to prove the correlation with the variability of the human immune system.

ACKNOWLEDGMENTS

Author superscripts are ordered by number for contribution and letter for alphabetical order by authors' name. Sophia Ying Li and Xiayan Li are the first co-authors (marked by plus sign), and Jiahe Gao, Kaiyang Pang, and Deyuan Xu are the second co-authors.

LIST OF FIGURES

4 MSA snapshot of protein N
5 Protein M frequency-based difference MSA
6 Protein M rasmol amino acid coloring

REFERENCES

"Coronavirus disease (COVID-19)."
"INSDC Country List."
"NCBI Multiple Sequence Alignment Viewer 1.21.0."
"WHO Coronavirus (COVID-19) Dashboard."
"WHO Director-General's opening remarks at the media briefing on COVID-19 - 11 March 2020."
A. T. Chen, K. Altschuler, S. H. Zhan, Y. A. Chan, and B. E. Deverman, "COVID-19 CG enables SARS-CoV-2 mutation and lineage tracking by locations and dates of interest," eLife, vol. 10, p. e63409, Feb. 2021.
A. Wilder-Smith, M. D. Teleman, B. H. Heng, A. Earnest, A. E. Ling, and Y. S. Leo, "Asymp- tomatic SARS Coronavirus Infection among Healthcare Workers, Singapore," Emerging In- fectious Diseases, vol. 11, pp. 1142–1145, July 2005.
B. Coutard, C. Valle, X. de Lamballerie, B. Canard, N. Seidah, and E. Decroly, "The spike glycoprotein of the new coronavirus 2019-nCoV contains a furin-like cleavage site absent in CoV of the same clade," Antiviral Research, vol. 176, p. 104742, Apr. 2020.
C. Wang, P. W. Horby, F. G. Hayden, and G. F. Gao, "A novel coronavirus outbreak of global health concern," The Lancet, vol. 395, pp. 470–473, Feb. 2020.
CDC, "COVID Data Tracker Weekly Review," Nov. 2021.
D. Planas, D. Veyer, A. Baidaliuk, I. Staropoli, F. Guivel-Benhassine, M. M. Rajah, C. Plan- chais, F. Porrot, N. Robillard, J. Puech, M. Prot, F. Gallais, P. Gantner, A. Velay, J. Le Guen,
H. Wang, X. Li, T. Li, S. Zhang, L. Wang, X. Wu, and J. Liu, "The genetic sequence, origin, and diagnosis of SARS-CoV-2," European Journal of Clinical Microbiology & Infectious Diseases: Official Publication of the European Society of Clinical Microbiology, vol. 39, pp. 1629–1635, Sept. 2020.
J. Kans, Entrez Direct: E-utilities on the Unix Command Line. National Center for Biotech- nology Information (US), Nov. 2021. Publication Title: Entrez Programming Utilities Help [Internet].
M. Chan, S. Vijay, J. McNevin, M. J. McElrath, E. C. Holland, and T. S. Gu- jral, "Machine learning identifies molecular regulators and therapeutics for targeting SARS[U+2010] CoV2[U+2010] induced cytokine release," Molecular Systems Biology, vol. 17, Sept. 2021.
N. Kassis-Chikhani, D. Edriss, L. Belec, A. Seve, L. Courtellemont, H. Pe´re´, L. Hoc- queloux, S. Fafi-Kremer, T. Prazuck, H. Mouquet, T. Bruel, E. Simon-Lorie`re, F. A. Rey, and O. Schwartz, "Reduced sensitivity of SARS-CoV-2 variant Delta to antibody neutraliza- tion," Nature, vol. 596, pp. 276–280, Aug. 2021.
R. C. Edgar, "[No title found]," BMC Bioinformatics, vol. 5, no. 1, p. 113, 2004.
R. McBride, M. van Zyl, and B. Fielding, "The Coronavirus Nucleocapsid Is a Multifunc- tional Protein," Viruses, vol. 6, pp. 2991–3018, Aug. 2014.
S. Kumar, G. Stecher, M. Li, C. Knyaz, and K. Tamura, "MEGA X: Molecular Evolutionary Genetics Analysis across Computing Platforms," Molecular Biology and Evolution, vol. 35, pp. 1547–1549, June 2018.
S. Satarker and M. Nampoothiri, "Structural Proteins in Severe Acute Respiratory Syndrome Coronavirus-2," Archives of Medical Research, vol. 51, pp. 482–491, Aug. 2020.
V. M. Corman, O. Landt, M. Kaiser, R. Molenkamp, A. Meijer, D. K. Chu, T. Bleicker,S. Bru¨nink, J. Schneider, M. L. Schmidt, D. G. Mulders, B. L. Haagmans, B. v. d. Veer, S. v. d. Brink, L. Wijsman, G. Goderski, J.-L. Romette, J. Ellis, M. Zambon, M. Peiris, H. Goossens, C. Reusken, M. P. Koopmans, and C. Drosten, "Detection of 2019 novel coronavirus (2019- nCoV) by real-time RT-PCR," Eurosurveillance, vol. 25, p. 2000045, Jan. 2020. Publisher: European Centre for Disease Prevention and Control.
X. Fang, J. Gao, H. Zheng, B. Li, L. Kong, Y. Zhang, W. Wang, Y. Zeng, and L. Ye, "The membrane protein of SARS-CoV suppresses NF-B activation," Journal of Medical Virology, vol. 79, pp. 1431–1439, Oct. 2007.
Y. Fan, K. Zhao, Z.-L. Shi, and P. Zhou, "Bat Coronaviruses in China," Viruses, vol. 11, p. 210, Mar. 2019.
Y. Huang, C. Yang, X.-f. Xu, W. Xu, and S.-w. Liu, "Structural and functional properties of SARS-CoV-2 spike protein: potential antivirus drug development for COVID-19," Acta Pharmacologica Sinica, vol. 41, pp. 1141–1149, Sept. 2020.
Y. Jung, G.-S. Park, J. H. Moon, K. Ku, S.-H. Beak, C.-S. Lee, S. Kim, E. C. Park, D. Park, J.-H. Lee, C. W. Byeon, J. J. Lee, J.-S. Maeng, S.-J. Kim, S. I. Kim, B.-T. Kim, M. J. Lee, and H. G. Kim, "Comparative Analysis of Primer–Probe Sets for RT-qPCR of COVID-19 Causative Virus (SARS-CoV-2)," ACS Infectious Diseases, vol. 6, pp. 2513–2523, Sept. 2020. Publisher: American Chemical Society.