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Models of spike, envelope and membrane proteins of SARS-CoV-2 variants for possible therapeutics

Olası terapötikler için SARS-CoV-2'nin spike, zarf ve membran proteinlerinin modelleri

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ABSTRACT

Background: SARS-CoV-2 associated with severe acute respiratory syndrome is a common, rapidly growing infectious disease with high morbidity and mortality. Mutations in the sequence of the three structural proteins of this virus may have a significant impact on the progression of the pandemic.
In this study, it was aimed to reveal possible targets for future drug and vaccine research by modeling and analyzing mutations in five variants of SARS-CoV-2 of concern.
Materials and Methods: RNA sequences of five variants identified by WHO as Variants of Concern (VoC) were obtained from NCBI. The translation of these sequences to amino acid sequence, sequence alignment and sequence comparison between variants was performed in RStudio 1.4.1717 with reference to 2020 study. Structural proteins and PDB files of the Wuhan variant were downloaded from studies conducted with the I-TASSER server. 3D representations of structural proteins were performed in LLC, Schrödinger, PyMOL Molecular Graphics System 1.2r3pre.
Results: The amino acid sequences of three structural proteins belonging to the VoC and the Wuhan lineage and the mutations are shown. The mutation numbers in Alpha, Beta, Delta and Gamma were as follows; 10, 8, 10 and 12. The envelope and membrane proteins had one mutation each in Beta and Delta, respectively.
Conclusions: The results show that several mutations occur the spike proteins of the SARS-CoV-2 variants. Only a small number of mutations are observed in envelope and membrane proteins. It may be beficial to focus on less mutated structural proteins for future vaccine and drug development studies.
Keywords: R programming language, SARS-CoV-2, Sequence alignment, Structural Proteins, Molecular Modelling

ÖZET

Amaç: Şiddetli akut solunum sendromu ile ilişkili SARS-CoV-2, yüksek morbidite ve mortaliteye sahip, yaygın, hızla büyüyen bir enfeksiyon hastalığıdır. Bu virüsün üç yapısal proteininin dizisindeki mutasyonlar, pandeminin ilerlemesi üzerinde önemli bir etkiye sahip olabilir.
Bu çalışmada, söz konusu SARS-CoV-2'nin beş varyantındaki mutasyonlar modellenip analiz edilerek, gelecekteki ilaç ve aşı araştırmaları için olası hedeflerin ortaya çıkarılması amaçlanmıştır.
Materyal ve Metot: DSÖ tarafından Endişe Verici Varyantlar (VoC) olarak tanımlanan beş varyantın RNA dizileri NCBI'den elde edildi. Bu dizilerin amino asit dizisine çevrilmesi, dizi hizalaması ve varyantlar arasında dizi karşılaştırması, 2020 çalışmasına referansla RStudio 1.4.1717'de gerçekleştirilmiştir.
Wuhan varyantının yapısal proteinleri ve PDB dosyaları, I-TASSER sunucusu ile yapılan çalışmalardan indirildi. Yapısal proteinlerin 3D görüntüleri LLC, Schrödinger, PyMOL Molecular Graphics System 1.2r3pre'de gerçekleştirilmiştir.
Sonuçlar: VoC ve Wuhan soyuna ait üç yapısal proteinin amino asit dizileri ve mutasyonlar gösterilmiştir. Alfa, Beta, Delta ve Gama'daki mutasyon sayıları ise şu şekildeydi; 10, 8, 10 ve 12. Zarf ve zar proteinleri sırasıyla Beta ve Delta'da birer mutasyona sahipti.
Sonuçlar: Sonuçlar, SARS-CoV-2 varyantlarının spike proteinlerinde birkaç mutasyon meydana geldiğini göstermektedir. Zarf ve zar proteinlerinde sadece az sayıda mutasyon gözlenir. Gelecekteki aşı ve ilaç geliştirme çalışmaları için daha az mutasyona uğramış yapısal proteinlere odaklanmak faydalı olabilir.
Keywords: R programlama dili, SARS-CoV-2, Dizi Hizalama, Yapısal Proteinler, Moleküler Modelleme

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INTRODUCTION

Coronaviruses are enveloped RNA viruses which are medium in size and have a crown-like appearance. These viruses have the longest viral RNA genomes known, ranging between 27 to 32 kb. The host-derived membrane is surrounded by glycolyzed spike proteins and is enclosed in a nucleocapsid that is helical when relaxed but takes on a spherical shape within the viral particle (Iannarella et al., 2020; McIntosh et al., 1967).

The novel coronavirus (SARS-CoV-2) which is associated with severe acute respiratory syndrome is a beta-coronavirus belonging to the Coronaviridae family, which is an RNA virus detected for the first time, in Wuhan, China, in December 2019 (Shamsi et al., 2021; Troyano-Hernáez et al., 2021). Since then, this newly identified virus has been named SARS-CoV-2 and has spread worldwide. It is considerably more infectious than previously identified human SARS-CoV (2002) and MERS-CoV (2013). Just like its' ancestors, SARS-CoV-2 as a member of the coronavirus family started by zoonotic (from animals to humans) transmission and is transmitted from person to person by respiratory tract and contact (Alsulami et al., 2021).

Spreading around the world so quickly, SARS-CoV-2 has also mutated in the regions where it spread, and some of these mutations included changes that caused the virus to peak again. These variants have been identified as Variants of Concern (VOCs) because of their more efficient binding to human cells, increased ability to evade the immune system, and rapid transmission (Scudellari, 2021). The four major variants; Alpha, Beta, Delta and Gamma which are reported first in the UK, South Africa, India and Brazil, in order and identified as Variants of Concern by WHO (*Classification of Omicron (B.1.1.529)*, n.d.; *Tracking SARS-CoV-2 Variants*, 2022). Finally, in late 2021, a new mutation originating from South Africa was identified by WHO as an alarming variant: Omicron (*Classification of Omicron (B.1.1.529)*, n.d.; Pulliam et al., 2021). Thus, there are five major variants listed as VoCs and subjected to this study.

The viral RNA of the Coronavirus (CoV) translates to main five structural proteins: S (Spike), M (Membrane), N (Nucleocapsid), HE (hemagglutinin-esterase glycoprotein), and E (Envelope) (Wormser & Aitken, 2010).

The nucleocapsid protein (N) binds to the RNA genome to create the nuclear envelope. It could be involved in regulating viral RNA synthesis and may interact with M protein during viral budding (Arabi et al., 2020; Omrani et al., 2014). The N protein, critical for viral genome packaging, contains three dynamically disordered regions that house conjectural temporally helical binding motifs. The two folded domains interact at a minimum level, making the full-length N protein a flexible and multivalent RNA-binding protein (Cubuk et al., 2021).

Although the SARS-CoV-2 E protein is a relatively small to other structural proteins, deletion of this protein weakens or even eradicates virulence. This is because the E protein is involved in many aspects of the viral lifecycle, such as promoting packaging and reproduction of the virus (Cao, Yang, et al., 2021). The short envelope (E) protein leaves its' C terminus from the inside of the envelope and then either spans the envelope or twists around and projects its' N terminus internally (Siu et al., 2008).

The most abounding structural protein of SARS-CoV-2 is the M glycoprotein (Buxbaum, 2015). The membrane (M) protein can bind to all other structural proteins. This protein contains a small N-terminal domain on the envelope's exterior surface and a long C terminus inside the envelope. The M protein is essential for viral assembly (Arabi et al., 2020). Binding with it promotes the stabilization of N proteins and the completion of viral assembly by the N protein-RNA complex within the internal virion (Mousavizadeh & Ghasemi, 2021). Since the M protein also cooperates with the S protein, mutations can affect the ability of host cell attachment (Buxbaum, 2015).

The spike (S) protein surrounds the viral envelope and forms the characteristic crown-like image. It is highly glycosylated and functions in receptor binding and fusion with the host cell membrane. The major antigens that stimulate neutralizing antibodies are on the S protein (Enjuanes et al., 1995). This structural protein has a total length of 1273 amino acids and a signal peptide (between 1–13) which is located at the N-terminus, the S1 (between 14–685) subunit and the S2 (between 686–1273) subunit. The S1 region is responsible for receptor binding and the S2 region is responsible for membrane fusion (Huang et al., 2020).

SARS-CoV and MERS-CoV are two very similar viruses in sequence, important structural proteins such as spike, envelope, membrane and nucleocapsid proteins are encoded in both. This similarity suggests that two similar viruses may have the same pathogenesis mechanisms that could be used for common therapeutic targeting (Shamsi et al., 2021). Therapeutic targeting of common regions and notably unmutated ones may represent a broad therapeutic approach (Li et al., 2020).

The 3D visualization of SARS-CoV-2 mutations in the S, E and M structural proteins which are located on the cell surface and have crucial roles in viral entry into the host cell, may assist in the understanding of the situation in general (Alsulami et al., 2021). It provides the opportunity to see molecular changes and their intensities. It also allows these representations of mutations in all variants to be compared, thus determining the most frequently mutated zones, so-called hotspots, is easier. Considering the rapid mutations of the virus since 2019, targeting the least mutated regions with small molecules may provide better therapeutic candidates and confidence in subsequent clinical trials.

MATERIALS AND METHODS

The RNA sequences of the variants' spike proteins were gathered from the National Centre for Biotechnology Information (NCBI), regarding the Variants of Concern which are determined by WHO (*Classification of Omicron (B.1.1.529)*, n.d.; *Tracking SARS-CoV-2 Variants*, 2022). Also, the researchers of this study surveyed the GISAID system for potential entries from Turkey which could be closely related to novel variants. As a result, a strain from Turkey selected and compared to variants of concern (Bayrakdar et al., 2021).

The translation process of the RNA to amino acid sequence, multiple sequence alignment and presentation of sequence comparison between variants' spike proteins were carried out in R, Version 4.0.3., by using the method from the study of Toparslan et al., 2020 and by the researchers of this study (Toparslan et al., 2020). The msa, ape, stats-package and gg-tree libraries have been used in R, for this analysis.

The file in FASTA format was read with the readDNAStringSet() command supported in the Biostrings package. With the msa() command supported in the msa package, all samples were aligned with the ClustaIW algorithm at the same size.

Sequences aligned with the phylogenetic tree are shown with the msaplot command supported in the ggtree and ggplot2 package. The geometric layers- geom_tiplab(), scale_color_continuous(), geom_tiplab(), geom_treescale() - supported in the ggplot2 packageare used to detail the tree.

We wish to demonstrate phylogenetic trees from multiple sequence alignments starting with an input file in FASTA format. Both R packages and R commands were helpful for all of these analysis and tables.

The Wuhan variant's structural proteins were used as a reference for visual comparison, and program database (PDB) files were downloaded from the in-silico studies conducted on I-TASSER server (Zhang et al., 2020; Zheng et al., 2021). These structures have been utilized to represent different mutations locally on the subunits.

3D representations of the structural proteins were carried out in PyMOL Molecular Graphics System, Version 1.2r3pre, Schrödinger, LLC. The 3D representations created considering the visibility aspects for the mutations. We aimed to highlight both subunits of interest and different mutations separately, so the mutation trends for the future variants could be understood clearly.

RESULTS

Amino acid sequences of three major structural proteins (S, E, and M) which are prominent in host cell entry and belong to the five VOCs and the Wuhan lineage were obtained from NCBI. Amino acid sequences and the mutations of these three proteins belonging to these five variants and the Wuhan lineage are analysed in this study.

Mutation counts, compared to Wuhan strain, including the deleted regions in the spike proteins, were on 12, 11, 10, 12 and 41 for Alpha, Beta, Delta, Gamma and Omicron in order. The mutual locations of these mutations among different variants are shown in the same colours in Figure 1. The envelope protein had a single mutation (P71L) in the Beta variant and another single mutation (T9I) in the Omicron variant. There was also a single mutation in the membrane protein in the Delta variant (I82T) and three mutations (D3G, Q19E, A63T) in the Omicron variant (Cao, Wang, et al., 2021; Troyano-Hernáez et al., 2021). Also, there has been a new entry in GSAID Initiative's EpiCoV on 2021/12/16 for a possible Omicron variant from Turkey with these aa substitutions in Spike protein; A67V, D614G, D796Y, G142D, G446S, H69del, H655Y, L981F, N679K, N764K,

N856K, N969K, P681H, Q954H, S477N, T95I, T478K, T547K, V70del, V143del, Y144del, Y145del and in Envelope protein; T9I and in Membrane protein; A63T, Q19E (Bayrakdar et al., 2021).

The obtained results are consistent with the mutations previously reported in the literature, and these structural proteins are considered important for possible drug targets (Portelli et al., 2020; Shen et al., 2021; Vilar & Isom, 2021). These mutations are shown on 3D models which are spike proteins (Figure 2), envelope proteins (Figure 3) and membrane proteins (Figure 4).

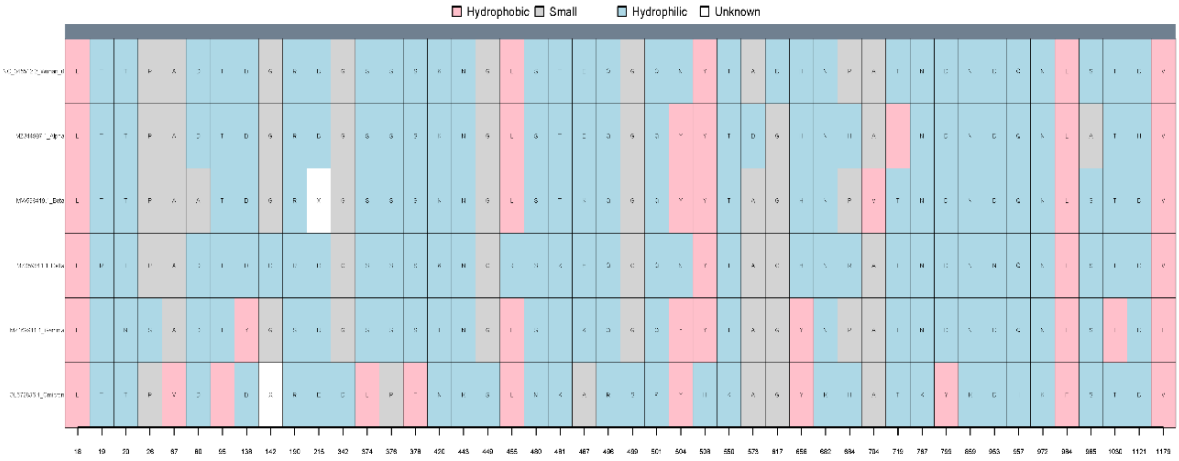

Figure 1. AA comparison of Spike proteins for the VoCs and Wuhan strain.

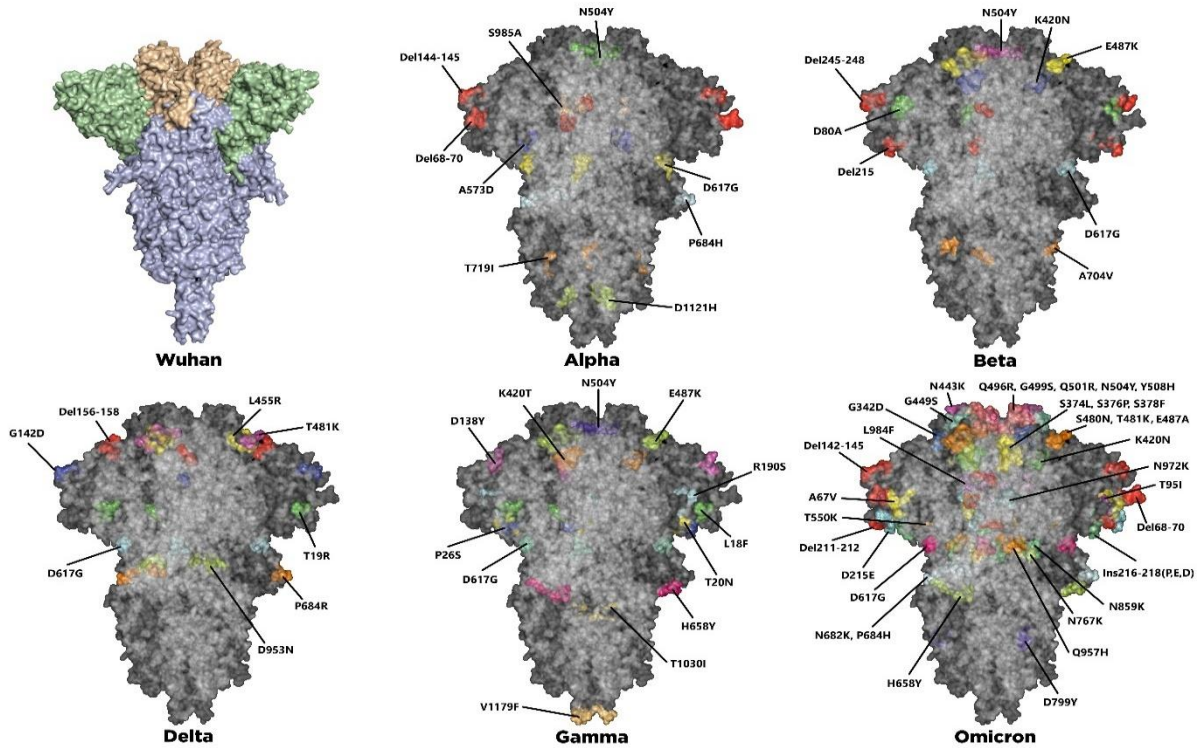

Figure 2. 3D models of Spike proteins for the VoCs and Wuhan Strain.

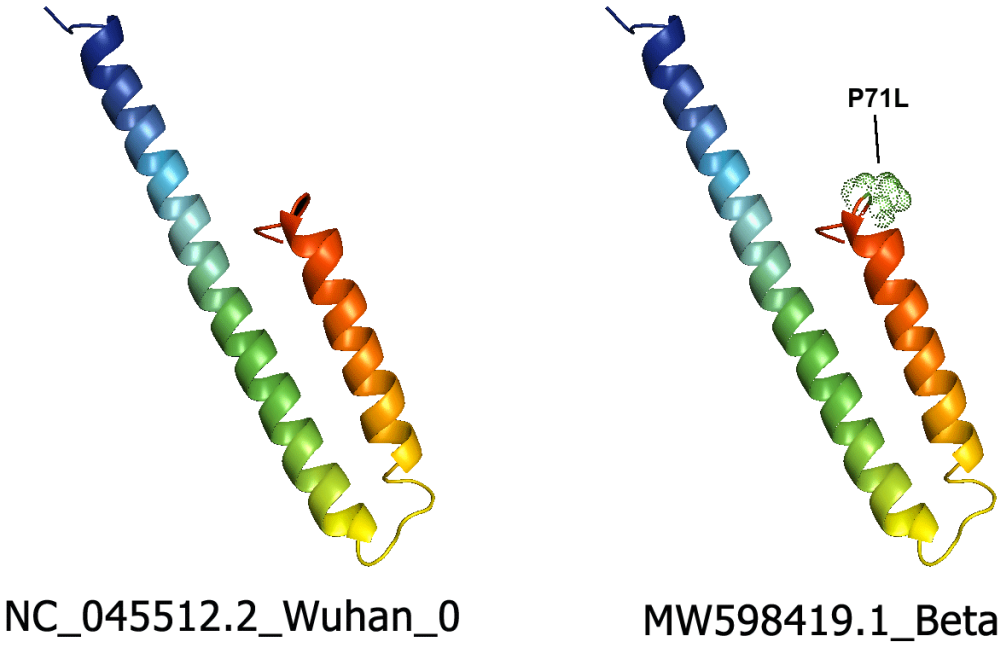

Figure 3. 3D models of Envelope proteins for the Beta variant and Wuhan strain.

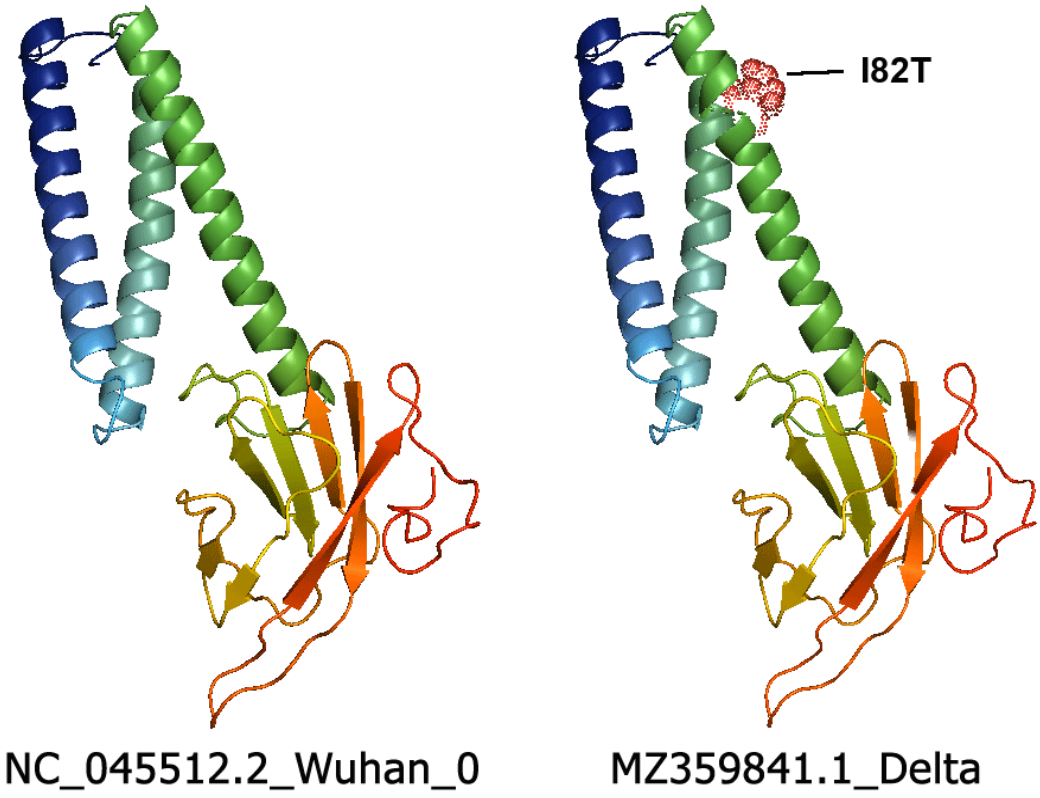

Figure 4. 3D model of Membrane proteins for the Delta variant and Wuhan strain.

DISCUSSION AND CONCLUSION

In silico studies can be advantageous in terms of reducing the financial burden and the total time spent (Good, 2020; Swain et al., 2021). In addition, 3D molecular models of SARS-CoV-2 and its' mutations facilitate understanding of the structural changes that occur. Thus, the distribution of these mutations reveals the most and the least frequently mutated regions. In addition, it also contributes to the prediction of changes in binding affinities. These changes in binding affinities and viral membrane fusion process can affect viral entry in the host.Also, these models can help determine target regions for future therapeutics such as drugs and vaccines (Kardani et al., 2020; Lee & Koohy, 2020). The models obtained from this study could point out potential target areas for future drugs that could be used on a large scale as mutation-resistant therapies (de Oliveira et al., 2021; Raghav et al., 2020). In a study, in silico analysis of the interaction between various RBD variants of SARS-CoV-2 pointed to a drug by called bromelain that effectively binds to the active site of RBD. This suggested the potential use of bromelain to prevent viral entry. In another study that performed binding free energy calculations and ADMET (adsorption, distribution, metabolism, excretion, and toxicity) studies predicting the stability and pharmacological potential of drug candidates, molnupiravir was predicted as one of the best inhibitors of the RNA-linked RNA polymerase of SARS-CoV-2.

In order to prevent the spread of the virus and to monitor the possible effects of vaccines, it is important to follow the changes in the SARS-CoV-2 genome and the variants that occur. To overcome the difficulty of this important task, the benefits of next generation genome sequencing technology should be utilized and its use should be widespread in such studies. A similar study used both network analysis and machine learning techniques to identify potentially significant sources of variation between orbitals. In another similar study, molecular insertion and molecular dynamics simulation against all variants of SARS-CoV-2 was used to validate the next-generation vaccine candidate developed and to understand its binding affinity to the immune receptor. In conclusion, because these representations include both sequence and molecular comparisons for viruses with comparable structures that belong to the coronavirus family, they could be of help for future studies that investigate novel variants of coronavirus which may represent a concern in future.

The results indicate that several mutations occurred in the spike proteins of SARS-CoV-2 variants, particularly in the S1 subunit, Receptor Binding Domain and Antibody Binding Domain. Spike proteins of all variants have many mutations while only a few changes are observed in envelope and membrane proteins. Both proteins have a single mutation in only one variant. For future studies regarding vaccine and drug development, it could be beneficial to focus on less mutated structural proteins.

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Ethics Committee Approval: This study is not required ethics committee approval due to conducting *in slico.*

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Conflict of Interest: The authors declare that there is no conflict of interest.

Author Contributions:

Conceptualization: Gizem TUTKUN**; Methodology:** Ahmet Ozan ÖZGEN; **Validation/Counsellorship:** Uğur BİLGE; **Data Collection and/or Processing:** Gizem TUTKUN, Ahmet Ozan ÖZGEN, Uğur BİLGE; **Analysis:** Gizem TUTKUN, Ahmet Ozan ÖZGEN; **Design of References:** Gizem TUTKUN, Ahmet Ozan ÖZGEN; **Design of Article:** Gizem TUTKUN, Ahmet Ozan ÖZGEN, Uğur BİLGE; **Writing:** Gizem TUTKUN**; Critical Review:** Uğur BİLGE

REFERENCES

Alsulami, A.F., Thomas, S.E., Jamasb, A.R., Beaudoin, C.A., Moghul, I., Bannerman, B., Copoiu, L., Vedithi, S.C., Torres, P. & Blundell, T.L. (2021). SARS-CoV-2 3D database: Understanding the coronavirus proteome and evaluating possible drug targets. Briefings in Bioinformatics, 22(2), 769–780. https://doi.org/10.1093/bib/bbaa404

Arabi, Y.M., Asiri, A.Y., Assiri, A.M., Balkhy, H.H., Al Bshabshe, A., Al Jeraisy, M., Mandourah, Y., Azzam, M. H. A., Bin Eshaq, A. M., Al Johani, S., Al Harbi, S., Jokhdar, H.A.A.,

Deeb, A.M., Memish, Z A., Jose, J., Ghazal, S., Al Faraj, S., Al Mekhlafi, G.A., Sherbeeni, N. M., … Saudi Critical Care Trials Group. (2020). Interferon Beta-1b and Lopinavir-Ritonavir for Middle East Respiratory Syndrome. The New England Journal of Medicine, 383(17), 1645–1656. https://doi.org/10.1056/NEJMoa2015294

Bayrakdar, F., Cosgun, Y., Yalcin, S. & Korukluoglu, G. (2021). HCoV-19/Turkey/HSGM-F10304/2021 (GISAID EpiCoV EPI_ISL_8082633). Ministry of Health Turkey.https://www.epicov.org/epi3/frontend#3f63b7

Buxbaum, E. (2015). Protein Structure. In E. Buxbaum, Fundamentals of Protein Structure and Function (pp. 15–64). Springer International Publishing. https://doi.org/10.1007/978-3-319-19920-7_2

Cao, Y., Wang, J., Jian, F., Xiao, T., Song, W., Yisimayi, A., Huang, W., Li, Q., Wang, P., An, R., Wang, J., Wang, Y., Niu, X., Yang, S., Liang, H., Sun, H., Li, T., Yu, Y., Cui, Q., … Xie, X.S. (2021). Omicron escapes the majority of existing SARS-CoV-2 neutralizing antibodies. Nature. https://doi.org/10.1038/d41586-021-03796-6

Cao, Y., Yang, R., Lee, I., Zhang, W., Sun, J., Wang, W. & Meng, X. (2021). Characterization of the SARS-CoV-2 E Protein: Sequence, Structure, Viroporin, and Inhibitors. Protein Science, 30(6), 1114–1130. https://doi.org/10.1002/pro.4075

Classification of Omicron (B.1.1.529): SARS-CoV-2 Variant of Concern. (n.d.). Retrieved 27 December 2021, from https://www.who.int/news/item/26-11-2021-classification-of-omicron-(b.1.1.529)-sars-cov-2-variant-of-concern

Cubuk, J., Alston, J.J., Incicco, J.J., Singh, S., Stuchell-Brereton, M.D., Ward, M.D., Zimmerman, M.I., Vithani, N., Griffith, D., Wagoner, J.A., Bowman, G.R., Hall, K.B., Soranno, A. & Holehouse, A.S. (2021). The SARS-CoV-2 nucleocapsid protein is dynamic, disordered, and phase separates with RNA. Nature Communications, 12(1), 1936. https://doi.org/10.1038/s41467-021-21953-3

de Oliveira, O.V., Rocha, G.B., Paluch, A.S. & Costa, L.T. (2021). Repurposing approved drugs as inhibitors of SARS-CoV-2 S-protein from molecular modeling and virtual screening. Journal of Biomolecular Structure and Dynamics, 39(11), 3924–3933. https://doi.org/10.1080/07391102.2020.1772885

Enjuanes, L., Smerdou, C., Castilla, J., Antón, I. M., Torres, J. M., Sola, I., Golvano, J., Sánchez, J.M. & Pintado, B. (1995). Development of protection against coronavirus induced diseases. A review. Advances in Experimental Medicine and Biology, 380, 197–211. https://doi.org/10.1007/978-1-4615-1899-0_34

Good, D.J. (2020). A low-cost, in silico nutritional genomics course-based undergraduate research experience applicable to multiple disciplines. Biochemistry and Molecular Biology Education, 48(4), 320–328. https://doi.org/10.1002/bmb.21352

Huang, Y., Yang, C., Xu, X., Xu, W. & Liu, S. (2020). Structural and functional properties of SARS-CoV-2 spike protein: Potential antivirus drug development for COVID-19. Acta Pharmacologica Sinica, 41(9), 1141–1149. https://doi.org/10.1038/s41401-020-0485-4

Iannarella, R., Lattanzi, C., Cannata, G., Argentiero, A., Neglia, C., Fainardi, V., Pisi, G. & Esposito, S. (2020). Coronavirus infections in children: From SARS and MERS to COVID-19, a narrative review of epidemiological and clinical features. Acta Biomedica Atenei Parmensis, 91(3), e2020032–e2020032. https://doi.org/10.23750/abm.v91i3.10294

Kardani, K., Bolhassani, A. & Namvar, A. (2020). An overview of in silico vaccine design against different pathogens and cancer. Expert Review of Vaccines, 19(8), 699–726. https://doi.org/10.1080/14760584.2020.1794832

Lee, C.H. & Koohy, H. (2020). In silico identification of vaccine targets for 2019-nCoV. F1000Research, 9, 145. https://doi.org/10.12688/f1000research.22507.2

Li, Y., Zhang, Z., Yang, L., Lian, X., Xie, Y., Li, S., Xin, S., Cao, P. & Lu, J. (2020). The MERS-CoV Receptor DPP4 as a Candidate Binding Target of the SARS-CoV-2 Spike. IScience, 23(6), 101160. https://doi.org/10.1016/j.isci.2020.101160

McIntosh, K., Dees, J.H., Becker, W.B., Kapikian, A.Z. & Chanock, R.M. (1967). Recovery in tracheal organ cultures of novel viruses from patients with respiratory disease. Proceedings of the National Academy of Sciences of the United States of America, 57(4), 933–940. https://doi.org/10.1073/pnas.57.4.933

Mousavizadeh, L. & Ghasemi, S. (2021). Genotype and phenotype of COVID-19: Their roles in pathogenesis. Journal of Microbiology,

Immunology and Infection, 54(2), 159–163. https://doi.org/10.1016/j.jmii.2020.03.022

Omrani, A.S., Saad, M.M., Baig, K., Bahloul, A., Abdul-Matin, M., Alaidaroos, A.Y., Almakhlafi, G.A., Albarrak, M.M., Memish, Z.A. & Albarrak, A.M. (2014). Ribavirin and interferon alfa-2a for severe Middle East respiratory syndrome coronavirus infection: A retrospective cohort study. The Lancet. Infectious Diseases, 14(11), 1090–1095. https://doi.org/10.1016/S1473-3099(14)70920-X

Portelli, S., Olshansky, M., Rodrigues, C.H.M., D'Souza, E.N., Myung, Y., Silk, M., Alavi, A., Pires, D.E.V. & Ascher, D.B. (2020). Exploring the structural distribution of genetic variation in SARS-CoV-2 with the COVID-3D online resource. Nature Genetics, 52(10), 999–1001. https://doi.org/10.1038/s41588-020-0693-3

Pulliam, J.R.C., Schalkwyk, C. van, Govender, N., Gottberg, A. von, Cohen, C., Groome, M.J., Dushoff, J., Mlisana, K. & Moultrie, H. (2021). Increased risk of SARS-CoV-2 reinfection associated with emergence of the Omicron variant in South Africa (p. 2021.11.11.21266068). https://doi.org/10.1101/2021.11.11.21266068

Raghav, S., Ghosh, A., Turuk, J., Kumar, S., Jha, A., Madhulika, S., Priyadarshini, M., Biswas, V. K., Shyamli, P. S., Singh, B., Singh, N., Singh, D., Datey, A., Avula, K., Smita, S., Sabat, J., Bhattacharya, D., Kshatri, J. S., Vasudevan, D., … Sherpa, T. (2020). Analysis of Indian SARS-CoV-2 Genomes Reveals Prevalence of D614G Mutation in Spike Protein Predicting an Increase in Interaction With TMPRSS2 and Virus Infectivity. Frontiers in Microbiology, 11, 2847. https://doi.org/10.3389/fmicb.2020.594928

Scudellari, M. (2021). How the coronavirus infects cells—And why Delta is so dangerous. Nature, 595(7869), 640–644. https://doi.org/10.1038/d41586-021-02039-y

Shamsi, A., Mohammad, T., Anwar, S., Amani, S., Khan, M. S., Husain, F. M., Rehman, Md. T., Islam, A. & Hassan, M. I. (2021). Potential drug targets of SARS-CoV-2: From genomics to therapeutics. International Journal of Biological Macromolecules, 177, 1–9. https://doi.org/10.1016/j.ijbiomac.2021.02.071

Shen, L., Bard, J.D., Triche, T.J., Judkins, A.R., Biegel, J.A. & Gai, X. (2021). Emerging variants of concern in SARS-CoV-2 membrane protein: A highly conserved target with potential pathological and therapeutic implications. Emerging Microbes & Infections, 10(1), 885–893.https://doi.org/10.1080/22221751.2021.1922097

Siu, Y.L., Teoh, K.T., Lo, J., Chan, C.M., Kien, F., Escriou, N., Tsao, S.W., Nicholls, J.M., Altmeyer, R., Peiris, J.S.M., Bruzzone, R. & Nal, B. (2008). The M, E, and N structural proteins of the severe acute respiratory syndrome coronavirus are required for efficient assembly, trafficking, and release of virus-like particles. Journal of Virology, 82(22), 11318–11330. https://doi.org/10.1128/JVI.01052-08

Swain, S.S., Rout, S.S., Sahoo, A., Oyedemi, S. O. & Hussain, T. (2021). Antituberculosis, antioxidant and cytotoxicity profiles of quercetin: A systematic and cost-effective in silico and in vitro approach. Natural Product Research, 0(0), 1–5. https://doi.org/10.1080/14786419.2021.2008387

Toparslan, E., Karabag, K. & Bilge, U. (2020). A workflow with R: Phylogenetic analyses and visualizations using mitochondrial cytochrome b gene sequences. PLOS ONE, 15(12), e0243927. https://doi.org/10.1371/journal.pone.0243927

Tracking SARS-CoV-2 variants. (2022, May 3). https://www.who.int/activities/tracking-SARS-CoV-2-variants

Troyano-Hernáez, P., Reinosa, R. & Holguín, Á. (2021). Evolution of SARS-CoV-2 Envelope, Membrane, Nucleocapsid, and Spike Structural Proteins from the Beginning of the Pandemic to September 2020: A Global and Regional Approach by Epidemiological Week. Viruses, 13(2), 243. https://doi.org/10.3390/v13020243

Vilar, S. & Isom, D. G. (2021). One Year of SARS-CoV-2: How Much Has the Virus Changed? Biology, 10(2), 91. https://doi.org/10.3390/biology10020091

Wormser, G.P. & Aitken, C. (2010). Clinical Virology, 3rd Edition Edited by D. D. Richman, R. J. Whitley, and F. G. Hayden Washington, DC: ASM Press, 2009. 1408 pp, Illustrated. $259.59 (hardcover). Clinical Infectious Diseases: An Official Publication of the Infectious Diseases Society of America, 50(12), 1692. https://doi.org/10.1086/652862

Zhang, C., Zheng, W., Huang, X., Bell, E.W., Zhou, X. & Zhang, Y. (2020). Protein Structure and Sequence Reanalysis of 2019-nCoV Genome Refutes Snakes as Its Intermediate Host and the Unique Similarity between Its Spike Protein

Insertions and HIV-1. Journal of Proteome Research, 19(4), 1351–1360. https://doi.org/10.1021/acs.jproteome.0c00129

Zheng, W., Zhang, C., Li, Y., Pearce, R., Bell, E. W. & Zhang, Y. (2021). Folding non-homologous proteins by coupling deep-learning contact maps with I-TASSER assembly simulations. Cell Reports Methods, 1(3), 100014. https://doi.org/10.1016/j.crmeth.2021.100014