Research Article

In Silico Analysis of Single Nucleotide Polymorphisms Related to Susceptibility and Severity in COVID-19 Patients

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Abstract

Coronavirus disease 2019 (COVID-19) is a respiratory tract symptom caused by the infection of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), which exhibits a wide range of symptoms, from mild to severe. Genetic factors play a significant role in determining the severity of the symptoms. Specifically, certain variants within genes can predispose individuals to experience severe COVID-19 symptoms. Therefore, this study aims to analyze these variants, also known as single nucleotide polymorphisms (SNP), within the ABO, TMPRSS2, ACE2, PAI-1, and IFNAR2 genes. The in silico method was done using internet-based softwares such as SIFT, Polyphen, SWISS-MODEL, and PyMol to identify the level of SNP pathogenicity, stability, visualization of 3-dimensional protein structures, and figures of amino acid changes, as well as to analyze the pathomechanism of variants that lead to susceptibility or severe symptoms of COVID-19 patients. This study was conducted from May to August 2023. The results showed that SNP influences changes in the structure or stability of proteins, and the 3-dimensional structure of all proteins affected by SNP was successfully visualized. Based on pathomechanism analysis and amino acid structure of F216I and P74S protein ABO residues, V160M protein TMPRSS2, I468V, and K26R protein ACE2 are associated with susceptibility of the patient to SARS-CoV-2 infection. In addition, the results of the in silico analysis showed that A15T residues of PAI-1 protein, F10V, and F8S IFNAR2 protein were included with pathomechanisms leading to severe symptoms of COVID-19.
Keywords: *COVID-19, Single Nucleotide Polymorphism (SNP), Amino Acid, Exon, Severity.*

Analisis *In Silico* Single Nucleotide Polymorphisms yang Terlibat dengan Kerentanan dan Gejala Berat Pasien COVID-19

Abstrak

Coronavirus disease 2019 (COVID-19) merupakan penyakit pada saluran pernapasan yang diakibatkan oleh infeksi severe acute respiratory syndrome corona virus 2 (SARS-CoV-2). Gejala yang ditimbulkan beragam dari ringan sampai berat. Salah satu faktor yang menyebabkan gejala berat ialah faktor genetik, yaitu adanya keterlibatan suatu varian pada gen yang menyebabkan seseorang mengalami kerentanan atau gejala berat COVID-19. Penelitian ini bertujuan menganalisis varian atau single nucleotide polymorphisms (SNP) dari gen ABO, TMPRSS2, ACE2, PAI-1, dan IFNAR2 dengan pendekatan in silico menggunakan perangkat lunak berbasis internet seperti SIFT, Polyphen, SWISS-MODEL, serta PyMol untuk mengidentifikasi tingkat patogenitas SNP, stabilitas, visualisasi struktur 3 dimensi protein, dan gambaran perubahan asam amino serta menganalisis patomekanisme varian yang mengarah kepada kerantanan atau gejala berat pasien COVID-19. Penelitian ini dilakukan sejak bulan Mei hingga Agustus 2023. Hasil penelitian menunjukkan bahwa keberadaan SNP berperan pada perubahan struktur atau stabilitas protein. Struktur 3 dimensi seluruh protein yang dipengaruhi oleh SNP telah berhasil divisualisasikan. Berdasarkan analisis patomekanisme dan struktur asam asam amino dari residu F216I dan P74S protein ABO, V160M protein TMPRSS2, I468V dan K26R protein ACE2 berkaitan dengan kerentanan pasien terhadap infeksi SARS-CoV-2. Selain itu, hasil analisis in silico kami menunjukkan bahwa residu A15T protein PAI-1, F10V dan F8S protein IFNAR2 terlibat dengan patomekanisme yang mengarah pada gejala berat COVID-19.
Kata kunci: *COVID-19, Single Nucleotide Polymorphism (SNP), Asam Amino, Ekson, Gejala Berat*

Introduction

Coronavirus disease 2019 (COVID-19) is a respiratory tract symptom caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection.1 The virus can manifest in various degrees of severity among infected patients, ranging from no symptoms, mild symptoms, severe symptoms, and even death.2 Several factors contribute to the likelihood of experiencing severe symptoms, such as age, gender, lifestyle, and comorbidity.[3] Additionally, previous study has identified genetic factors contributing to severe symptoms. The results of previous studies found that there were several gene variants found to be included with susceptibility and severe symptoms of patients, including rs8176740 and rs512770 ABO genes, rs1239760 TMPRSS2 genes, rs191860450 and rs4646116 in ACE2 genes, rs6092 PAI-1 genes, rs1051393 and rs2229207 IFNAR2 genes[4–8]

This study discovered an association between genetic variants in specific genes and the condition of COVID-19 patients. These variants, also known as nucleotide polymorphism (SNP), are genetic variations found within a population and in the genome.[9] Subsequently, changes in nitrogenous bases within the exon region of the genome result in an alternation in amino acid changes and give rise to genetic variations known as non-synonymous SNP. These genetic variations contribute to the wide range of functions performed by proteins encoded in the human body, affecting biological processes and serving as a biomarker for symptoms.[10,11] Therefore, SNP has an important role in determining an individual susceptibility to particular symptoms, one of which is COVID-19.[12]

Previous investigations reported an association between variants genes such as the ABO, TMPRSS2, ACE2, SERPINE1, and IFNAR2 and susceptibility to or severity of symptoms of COVID-19 patients. Prior to this, there have been no earlier reports on the in silico investigation of variations or single nucleotide polymorphisms (SNPs) in these genes that cause severe susceptibility or symptoms in COVID-19 patients. Therefore, the objective of this study is to present the findings of the in silico analysis, including SNP pathogenicity levels, protein stability assessment, identification of conserved sites, visualization of 3-dimensional protein structures, and the pictures of amino acid structures due to the presence of SNP in ABO, TMPRSS2, ACE2, SERPINE1 genes concerning susceptibility or severe symptoms of COVID-19 patients.

Methods

In this study, SNP data on genes associated with susceptibility and severe symptoms of COVID-19 patients were obtained through literature reviews. Subsequently, eight SNP were obtained from the ABO, TMPRSS2, ACE2, SERPINE1, and IFNAR2 genes. For the ABO gene, there are two variants, rs8176740 (F216I) and rs512770 (P74S); TMPRSS2 gene, rs12329760 (V160M); ACE2 genes, rs191860450 (I468V) and rs4646116 (K26R); PAI-1 rs6092 (A15T); and IFNAR2 genes, rs1051393 (F10V) and rs2229207 (F8S) variants.

To assess the pathogenicity of these SNP, various bioinformatics tools were used, including Sorting Intolerant From Tolerant (SIFT) (https://sift.bii.a-star.edu.sg/), Polymorphism Phenotyping v2 (PolyPhen 2.0) (http://genetics.bwh.harvard.edu/pph2/), Predictor of Human Deleterious Single Nucleotide Polymorphisms (PhD SNP) (https://SNP.biofold.org/phd-snp/phd-snp.html,SNP&GO (https://SNP.biofold.org/SNP-and-go/SNP-and-go.html),SNAP2(https://rostlab.org/services/snap2web/), and Functional Analysis through Hidden Markov Models (FATHMM) (https://fathmm.biocompute.org.uk/). Furthermore, to determine the stability of proteins, MuPro bioinformatics tools (http://mupro.proteomics.ics.uci.edu/) and I. Mutant Suite (https://folding.biofold.org/i-mutant//pages/I-Mutant2.0_Tutorial.html) were used.[12] These tools assessed whether amino acid changes within the protein structures increased or decreased stability.

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Cumulative Score of SNP Pathogenicity Rate

The prediction of amino acid changes effect was carried out on all bioinformatics tools (SIFT, PolyPhen 2.0, PhD SNP, SNP&GO, SNAP2, FATHMM, MuPro, and I. Mutant Suite 2.0) by summing all scores in Microsoft Excel. A value of 1 was assigned if SNP results of the SNP test show not tolerated/effect/damaging/symptoms, and a value of 0 if tolerated/benign/neutral.[12]

Conserved Domain Analysis on Protein Sequences

Conserved Domain Analysis on the protein sequences was performed using the Conserved Domain Database (CDD), accessible at (https://www.ncbi.nlm.nih.gov/cdd). Protein sequences were entered into queries contained at CDD sites, and then CDD will analyze the conserved sites on those sequences based on the multiple sequence arrangement method. The protein sequence will then be compared with the consensus sequence, and the conserved site of the protein sequence will be known.

Post-translational Modification Site Analysis

Site analysis of post-translational modification of phosphorylation, acetylation, ubiquitination, and methylation was carried out using several internet-based devices such as NetPhos, NetOGlyc-4.0, NetNGlyc-1.0 (https://services.healthtech.dtu.dk), GPS (http://gps.biocuckoo.cn/), GPS-MSP 1.0 (http://msp.biocuckoo.org/), GPS-PAIL (http://pail.biocuckoo.org/).

Protein 3-Dimensional Structure Modeling

Using homology modelling methods, the 3-dimensional structure modelling of proteins was carried out using the internet-based device SWISS-MODEL (https://swissmodel.expasy.org). In FASTA format, protein sequences subjected to SNP changes were inputted as queries, prompting SWISS-MODEL to perform automatic modelling based on protein templates sharing sequence similarities with the provided data. Subsequently, the modelling results matched the structure with the WT protein structure obtained from the Protein Data Bank (https://www.rcsb.org/) and the AlphaFold Protein Structure Database (https://alphafold.ebi.ac.uk/).

Aligned structures were carried out using PyMol to visualize changes in the structure of proteins that experienced SNP changes compared to the WT protein. Subsequently, the effect of protein structure changes due to gene variants was analyzed using HOPE (Have (y)Our Protein Explained), accessed at https://www3.cmbi.umcn.nl/hope/. HOPE is a website-based tool used to analyze a single mutation point, where the device collects data sources and results in the form of images illustrating the point of mutation.

Results

SNP Effect Prediction

The pathogenicity effect analysis of SNP using the SIFT, PolyPhen 2.0, PhD SNP, SNP & GO, SNAP2, and FATHMM devices show the results of the pathogenicity level of each SNP with various results, ranging from not tolerated/benign/neutral to experiencing the effects of not tolerated/damaging/symptoms/effect (Table 1).

Table 1. The Result of The Effects of SNP Prediction

Genes	Amino Acid	SIFT	PolyPhen 2.0	PhD SNP	SNP&GO	SNAP2	FATHMM
ABO	F216I	Not Tolerated	Pro Dam	Symptoms	Symptoms	Effect	Tolerated
ABO	P74S	Not Tolerated	Pos Dam	Neutral	Neutral	Neutral	Tolerated
TMPRSS2	V160M	Not Tolerated	Pro Dam	Symptoms	Neutral	Effect	Tolerated
ACE2	I468V	Tolerated	Pro Dam	Neutral	Neutral	Neutral	Tolerated
ACE2	K26R	Tolerated	Benign	Neutral	Neutral	Neutral	Tolerated
PAI-1	A15T	Tolerated	Benign	Neutral	Neutral	Neutral	Damaging
IFNAR2	F10V	Tolerated	Benign	Neutral	Neutral	Effect	Tolerated
IFNAR2	F8S	Tolerated	Benign	Neutral	Neutral	Effect	Tolerated

Pro Dam: probably damaging; Pos Dam: possibly damaging

Effect of Protein Stability

Table 2 shows the protein stability effect in the presence of amino acid changes using MuPro and I. Mutant Suite. The results of the I. Mutant Suite and MuPro obtained all proteins (F216I, P74S, V160M, I468V, K26R, A15T, F10V, F8S) due to SNP decreased protein stability.

Cumulative Scoring Results

Based on the calculation results of all eight algorithms (Table 4), F216I amino acid residues have the highest level of deleterious effects, with a score of 6. The V160M amino acid residues have a score of 5, while I468V, F10V, and F8S amino acid residues have a score of 4. Furthermore, amino acid residues with a score of 3 are owned by P74S, K26R, and A15T.

Table 2. The Result of Protein Stability Change Prediction Using I. Mutant Suite and MuPro

	I. Mutant Suite		MuPro	
Amino Acid	Stability	DDG value (kcal/mol)	Prediction	Delta G
F216I	Decrease	-1,64	Decrease	-0,934
P74S	Decrease	-1,72	Decrease	-1,196
V160M	Decrease	-1,58	Decrease	-0,964
I468V	Decrease	-0,7	Decrease	-0,988
K26R	Decrease	-0,34	Decrease	-0,396
A15T	Decrease	-1,15	Decrease	-1,462
F10V	Decrease	-2,33	Decrease	-1,109
F8S	Decrease	-2,29	Decrease	-2,329

DDG: the free energy change value

Table 3. The Result of Cumulative Scoring of SNP Effect Prediction

Mutation	SIFT	PolyPhen 2.0	PhD SNP	SNP&GO	SNAP2	FATHMM	I. Mutant	MuPro	Total
F216I	0	1	1	1	1	0	1	1	6
P74S	0	1	0	0	0	0	1	1	3
V160M	0	1	1	0	1	0	1	1	5
I468V	1	1	0	0	0	0	1	1	4
K26R	1	0	0	0	0	0	1	1	3
A15T	1	0	0	0	0	1	1	1	4
F10V	1	0	0	0	1	0	1	1	4
F8S	1	0	0	0	1	0	1	1	4

Conserve Domain Analysis

Based on analysis performed on each protein using the Conserve Domain Database (www.ncbi.nlm.nih.gov/Structure/cdd/cdd.shtml). The analysis examined numerous sequence alignments, revealing specific amino acid modifications at conserved locations.These changes include F216I and P74S in the ABO protein, V160M in the TMPRSS protein, and F10V & F8S in the IFNAR2 protein. Amino acid changes K26R & I468V in the ACE2 protein, and A15T in the PAI-1 protein is not located at the conserved site. The results of the analysis can be observed in the figure.

Post-Translational Modification Site Predictions

Based on analysis of post-translational modification sites using NetPhos, GPS, NetOGly, and NetNGly, it has been determined that variations of F216I and P74S in ABO proteins are not located at phosphorylation, glycosylation, or methylation sites, thereby there is no change in structure at these sites. Similarly, changes in amino acid V160M in the protein TMPRSS2, K26R and I468V in the ACE2 protein, A15T in the PAI-1 protein are not located at the post-translational sites of phosphorylation, methylation, and O-glycosylation. Amino acid F10V and F8S changes are not located at phosphorylation, methylation, and glycosylation sites.

The 3D Modelling and Analysis of Protein Structure

The 3-dimensional structure of proteins was visualized by Swiss-MODEL (https://swissmodel.expasy.org/). The generated protein model was formed based on protein structure templates obtained from the Protein Data Bank database and AlphaFoldDB v4. The modelling results are shown in Figure 1. The results are evaluated using the Qualitative Model Energy Analysis (QMEAN), as shown in Table 4. The QMEAN value obtained in each modelling result is quite good, which is 0.64 to 0.9, where a value close to 1 shows the quality of a good model structure.

The modelling results were then matched with the wild-type protein structure to scrutinize the impact of SNP on changes in the protein structure. The arrangement results are assessed based on the Root Mean Squared Deviation (RMSD) value, where the higher RMSD value shows a significant structural difference between the two matched proteins. The results of the arrangement of mutant and wild-type proteins (Figure 1) show no significant structural differences, with a relatively small RMSD value obtained around 0.5. To gain a deeper comprehension of the effects of alterations in the amino acid residue on the structure and function of the protein, the internet-based toolbox developed by Project HOPE was employed.

Table 4. Modelling and Superimposing of Protein Structure

Protein	Position	Template	Reference	QMEAN/GMQE	RMSD
ABO	F216I	4Y63	Protein Data Bank	0,9 ± 0,5	0,044
ABO	P74S	4Y63		0,9 ± 0,5	0,043
TMPRSS2	V160M	7MEQ		0,64 ± 0,5	0,092
ACE2	I468V	7Y75		0,84 ± 0,5	0,113
ACE2	K26R	7Y75		0,83 ± 0,5	0,118
PAI-1	A15T	P50449	AlphaFold V4	0,90	0,053
IFNAR2	F10V	P48551		0,64	0,054
IFNAR2	F8S	P48551		0,64	0,054

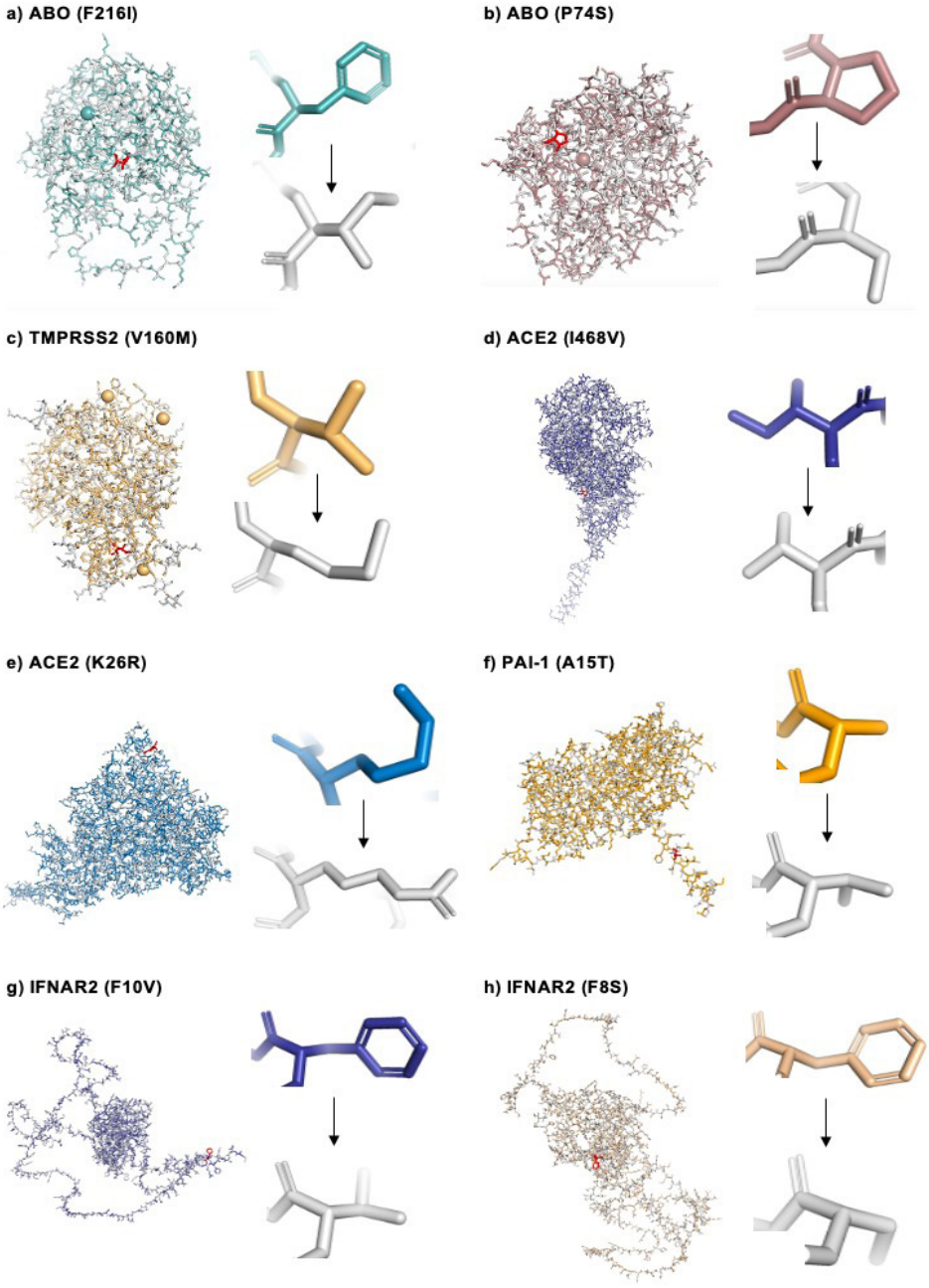
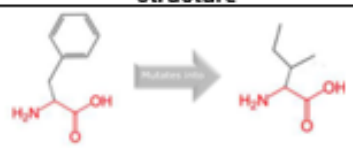
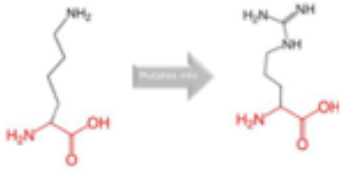


Figure 1. Aligned structure of Wild-Type and Mutant Models of Proteins.
A). Visualization of amino acid change 216th position, Phenylalanine to Isoleucine (F216I); b). Change of amino acid 74th position, Porlin to Serine (P74S); c). Change of amino acid 160th position, Valine to Methionine (V160M); d). Change of amino acid 468th position, Isoleucine to Valine (I468V); e). Changes in amino acid of the 26th position, Lysine to Arginine (K26R); f). Change of amino acid of the 15th position, alanine to threonine (A15T); g). Change of 10th position amino acid, Phenylalanine, to Valine (F10V); h). Change of amino acid 8th position, Phenylalanine to Serine (F8S).

Table 5. Identification of The Amino Acid Residue Change Effect Using The HOPE Project

Protein	Position	Structure	Properties
ABO	F216I		• The size of the mutant residue is less than that of the wild-type residue. • The amino acid alterations are situated in the glycosyltransferase 6 domain.
ABO	P74S		• The mutant residue is a polar amino acid, whereas the wild-type residue is a non-polar amino acid. • The amino acid alterations are situated in the glycosyltransferase 6 domain. Alterations in hydrophobicity could potentially impact protein interactions.
TMPRSS2	V160M		• The mutation is inside the scavenger receptor cysteine-rich (SRCR) domain. • The mutation resulted in an alteration in the amino acid sequence, which can disrupt the characteristics and function of this domain.
ACE2	I468V		• The mutant residue is smaller in size compared to the wild-type residue. • The mutant residue is smaller in size compared to the wild-type residue. • The mutation will result in a void in the protein's core.
ACE2	K26R		• The mutant residue is larger than the wild-type residue. • The residue is situated on the protein's surface, and mutation of this residue can disrupt its interactions. • The mutation is situated in the peptidase M2 domain
PAI-1	A15T		• The mutant residue is larger than the wild-type residue in size. • The hydrophobicity of the mutant and wild-type residues is not the same. • The alteration in the residue is situated inside the signal peptide.
IFNAR2	F10V		• The mutant residue is reduced in size, potentially resulting in the loss of interactions. • The mutation is situated inside the signal peptide.

Discussion

COVID-19 is a respiratory tract symptom caused by the infection of the SARS-CoV-2 virus. Genetic factors play a role in influencing the risk and severity of COVID-19 symptoms. Results of previous studies show that some SNP within the gene, such as *ABO*, *TMPRSS2*, *ACE2*, *PAI1*, and *IFNAR2* genes had associations with susceptibility

or severe symptoms of COVID-19 patients. This study conducted a comprehensive analysis of SNP from various genes using multiple bioinformatics tools. The primary objectives were to assess the impact of deleterious SNP, evaluate protein stability, examine conserved domains, visualize the 3-dimensional protein structures, and illustrate changes in amino acid structures resulting from SNP. The identification of the effect of SNP on protein structure was carried out in previous studies with different variants, such as the investigation conducted by Saih[12] and Paniri[13], who conducted in silico analysis on the ACE2 gene variants, Salleh and Deris[14] on TMPRSS2 gene variants, and Akter)[15] on IFNAR2 gene variants.

Saih[12] previously analyzed amino acid changes using several bioinformatics tools such as SIFT, Provean, PolyPhen 2.0, and Panther.[12] In this study, the identification of the effects caused by SNP was carried out using several bioinformatics tools, including SIFT, PolyPhen 2.0, SNAP2, PhD SNP, and SNP &GO, as well as I. Mutant Suite and ModPred to analyze the protein stability. The findings of SNP effect analysis showed that each bioinformatics tool demonstrated different results. The accumulative scores on all algorithms found that SNP with the highest pathogenicity was rs8176740 ABO protein. Subsequently, protein stability analysis with SNP variations rs8176740, rs512770, rs12329760, rs191860450, rs4646116, rs6092, rs1051393, and rs2229207 showed a decrease in stability. Alterations in amino acids in protein structure can affect conformational changes, salt bridges, protein interactions, and disruption of hydrogen bonding.[16]

Analysis identified conserved domain sites within each protein sequence that had experienced amino acid changes. Subsequently, alterations in amino acid residues, such as F216I and P74S in ABO proteins, V160M in TMPRSS proteins, as well as F10V and F8S in IFNAR2 proteins were located at conserved sites. Meanwhile, amino acid K26R and I468V changes in the ACE2 protein and A15T from the PAI-1 protein are not located at the conserved site. Conserved sites in protein sequences are regions that tend to remain unchanged throughout evolution. These regions often play crucial roles in protein function, including potential defense functions such as ligand binding functions, enzymatic processes, interactions between proteins, and defense of the shape of the protein structure.

In addition to the analysis of conserved domain sites, post-translational modification sites are important for investigating protein functional change. Some common post-translational modification processes in proteins include phosphorylation, acetylation, ubiquitination, methylation, and glycosylation.[17] The identification of post-translational modification sites using several internet-based bioinformatics tools shows that changes in amino acid residues that occur due to SNP variations rs8176740, rs512770, rs12329760, rs191860450, rs4646116, rs6092, rs1051393, and rs2229207 are not found at the post-translational modification sites. Therefore, it may not impact structural changes, including post-translational modification processes.

When visualizing and arranging the 3-dimensional structure of mutant proteins with wild-type proteins, no alterations in the secondary structure were observed due to changes in amino acid residues caused by SNP. The RMSD value shows that ACE2 proteins with changes in amino acid residues I468V (0.113) and K26R (0.118) have more influential changes when compared to proteins and changes in other amino acid with RMSD values <0.1. Although no changes in secondary structure have been observed, changes in an amino acid are known to affect the stability and its interaction with other proteins.[18]

Based on the results of analysis using the HOPE, amino acid changes in F216I and P74S residues occur in the glycosyltransferase6 domain. Subsequently, glycosyltransferase is an enzyme that plays a role in synthesizing antigens A and B in the blood group system. Polymorphisms influence the ABO blood group system in the ABO gene. Based on previous studies, blood type A is susceptible to SARS-CoV-2 compared to blood type O. This is due to the presence of anti-A antibodies in blood type O; the bond between the spike protein in the virus and the ACE2 receptor becomes inhibited.[19] SNP rs8176740 and rs512770 ABO genes are associated with high platelet levels and are at risk of developing COVID-19 symptoms. The mechanism of platelet levels associated with SNP rs8176740 is still unclear.[4] However, other mechanisms suggest that SARS-CoV-2 infection and replication in lung Megakaryocytes can promote platelet production in COVID-19 patients.[20]

The results of the HOPE project analysis reported certain changes in amino acid V160M in the TMPRSS2 protein located in the cysteine-rich receptor scavenger domain (SRCR). SRCR is a protein domain that binds proteins to cell surfaces and other extracellular molecules.[21] The impact of residual changes on the SRCR domain of TMPRSS2 proteins remains unknown. However,

studies on proteins within the same family, such as TMPRSS3, have shown that residual changes in the SRCR domain can decrease proteolytic activity.[22] Decreased proteolytic activity may affect the interaction of TMPRSS2 proteins with SARS-CoV-2, which is related to the symptoms experienced by the patient. However, there is a need for this to be confirmed through further investigation. The results of the in silico analysis study are in accordance with the research conducted by Wulandari[5], where the presence of SNP rs12329760 (V160M) gene TMPRSS2 is not associated with severe symptoms. This is following the study of Hou[23], which stated that SNP rs12329760 is associated with susceptibility to SARS-CoV-2 infection.

The results of other analyses with the HOPE project showed changes in amino acid I486V in the ACE2 protein, causing the formation of space in the protein nucleus and the size of the mutant amino acid to be smaller. This follows an analysis of Bakhshandeh[1], which reported that changes in amino acid valine with a smaller size cause protein instability. Additionally, alterations in the K26R residues within the ACE2 protein are situated in the M2 peptidase domain, potentially disrupting the functionality of that domain. M2 peptidase is a conserved domain that plays a role in the cleavage of bonds between peptides.[24] The peptidase domain in ACE2 is also known to function for SARS-Cov-2 protein S and receptor binding.[6] The results of molecular docking studies show that SNP rs4646116 (K26R) caused an increase in receptor affinity for the SARS-CoV-25 spike protein.[25] This may facilitate the bond of SARS-CoV-2 to the ACE2 receptor, and as a result, the presence of ACE2 gene variants is associated with susceptibility to SARS-CoV-2 infection.[25,26]

Analysis was carried out using the HOPE project, and the signalP (https://services.healthtech.dtu.dk/services/SignalP-6.0/) site showed changes in amino acid A15T protein PAI-1 located at the peptide signal site. Subsequently, peptide signalling sites play a role in targeting and translocating proteins. The change of amino acid from alanine to threonine, which is hydrophilic and polar, causes the peptide signalling function to be disrupted.[27] The PAI-1 gene encodes plasminogen activator inhibitor 1 (PAI-1) secreted in vascular endothelial cells. Based on the results of the SNP rs6092 study, the PAI-1 gene has an association with severity in COVID-19 patients, which is characterized by increased plasma levels of PAI-1.[7] The PAI-1 protein is recognized for its role in the blood clotting process (thrombosis) as it inhibits the activation of plasminogen such as urokinase (uPA) and tissue-type plasminogen activator (tPA). These activators convert plasminogen into plasmin, essential for breaking down blood clots through fibrinolysis. Therefore, if there is secretion of PAI-1 in endothelial cells, it could potentially inhibit the process of fibrinolysis in COVID-19 patients.[28] The results of the HOPE project analysis showed that changes in amino acids located on the peptide signal also occurred in F10V and F8S residues on the IFNAR2 protein. Changes in phenylalanine residues to serine, which are more polar, can impact the function of the signal peptide because the signal peptide domain is typically hydrophobic. These alterations may affect the hydrophobic-hydrophilic balance critical for proper signal peptide function.[29] Subsequently, IFNAR2 is a receptor that plays a role in antiviral activity. IFNAR2 receptors bind to interferon ligands (IFN), activate the Janus kinase (JAK) pathway, and induce genes that play a role in antiviral activity. Based on previous studies, the rs1051393 (F10S) and rs2229207 variants of the IFNAR2 gene cause COVID-19 patients to develop severe symptoms.[8] This is due to the weakening of the bond between IFN and IFNAR2 receptors; thereby, the antiviral activity decreases.[30]

Conclusion

In conclusion, the in silico study results on the effects of SNP and visualization of 3D protein structures showed that the presence of SNP affected the protein structure and its stability caused by differences in type, size, and polarity of amino acid residues. Pathomechanism analysis and description of the residual structure of F216I &; P74S protein ABO, V160M protein TMPRSS2, I468V &; K26R protein ACE2 were related to patient susceptibility to SARS-CoV-2 infection. In addition, changes in amino acid residues of A15T protein PAI-1, F10V &; and F8S protein IFNAR2 were related to pathomechanisms that led to severe symptoms in COVID-19 patients.

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