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In Silico Study of Splicing Variations on Angiotensin-Converting Enzyme 2 (ACE2) and Its Effects on Infection from SARS-CoV-2

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Several factors can influence SARS-CoV-2 infection. Alternative Splicing is thought to correlate with the differences of affinity value for interactions with the SARS-CoV-2 spike protein so that the infection may be influenced. This phenomenon can be happened by generating various kinds of protein expression variations with different isoform variants. So far, the difference in interaction affinity with the SARS-CoV-2 spike protein in Alternative Splicing has not been widely reported. Therefore, this study aimed to determine the isoform resulting from Alternative Splicing in the ACE2 transcript and its effect on SARS-CoV-2 infection. Molecular docking was used to determine the binding affinity between ACE2 proteins isoforms and SARS-CoV-2 spike protein. The 3D protein model of ACE2 isoforms obtained from the database was validated by evaluating the model quality of each ACE2 isoform. Based on docking results, there are variations in the docking scores of 8 isoform variants. However, there was no interaction between the ACE2_205 variant and SARS-CoV-2 spike protein while ACE2_206 and ACE2_207 showed a lower docking score than the other variants. In addition, essential residues in the interaction of each variant were also analyzed. Q42 and A384 are residues on the ACE2 protein that appear in interactions in more than two variants. These results indicate the possibility that splicing variations can cause differences in a person's level of susceptibility to SARS-CoV-2 infection, especially in the ACE2_205 variant that cannot interact with the SARS-CoV-2 spike protein.

Key words: ACE2, Alternative Splicing, Molecular Docking, SARS-CoV-2, Susceptibility

Beberapa faktor dapat mempengaruhi infeksi SARS-CoV-2. *Alternative Splicing* diduga berkorelasi dengan perbedaan nilai afinitas interaksi dengan protein *spike* SARS-CoV-2 sehingga dapat mempengaruhi infeksi. Fenomena ini dapat terjadi dengan menghasilkan berbagai macam variasi ekspresi protein dengan varian isoform yang berbeda. Sejauh ini, perbedaan afinitas interaksi dengan protein *spike* SARS-CoV-2 dalam *Alternative Splicing* belum banyak dilaporkan. Oleh karena itu, penelitian ini bertujuan untuk mengetahui isoform yang dihasilkan dari *Alternative Splicing* pada transkrip ACE2 dan pengaruhnya terhadap infeksi SARS-CoV-2. *Molecular Docking* digunakan untuk menentukan afinitas pengikatan antara protein isoform ACE2 dan protein *spike* SARS-CoV-2. Model protein 3D isoform ACE2 yang diperoleh dari database divalidasi dengan mengevaluasi kualitas model masing-masing isoform ACE2. Analisis *docking* menunjukkan variasi *docking score* dari 8 varian isoform. Varian ACE2_205 ditemukan tidak memiliki interaksi dengan protein *spike* SARS-CoV-2. Varian ACE2_206 dan ACE2_207 menunjukkan skor docking yang lebih rendah dibandingkan varian lainnya. Selain itu, residu esensial dalam interaksi masing-masing varian juga dianalisis. Residu Q42 dan A384 adalah residu pada protein ACE2 yang muncul dalam interaksi di lebih dari dua varian. Hasil ini menunjukkan kemungkinan bahwa variasi splicing dapat menyebabkan perbedaan tingkat kerentanan seseorang terhadap infeksi SARS-CoV-2, terutama pada varian ACE2_205 yang tidak dapat berinteraksi dengan protein *spike* SARS-CoV-2.

Kata kunci: ACE2, *Alternative Splicing*, Kerentanan, *Molecular Docking*, SARS-CoV-2

The COVID-19 pandemic has reached 152 million active cases as of May 2, 2021, according to a report released by the World Health Organization (WHO 2021). This high number is due to the high rate of infection of SARS-CoV-2, the virus that causes COVID-19. Factors that influence the infection rate of this virus include temperature, age, humidity, behaviour, and genetics (Abduljabbar *et al.* 2020; Davies *et al.* 2020; Liu *et al.* 2020).

The correlation between the increased of the SARS-CoV-2 virus infection rate and genetics has been previously studied through the SNP phenomenon. SNP is a form of genetic variation caused by a single base change. In this case, it has been studied through the SNP present at the receptor that plays a role in viral interactions, namely ACE2 in humans. The study showed differences in the binding affinity of ACE2 protein to the SARS-CoV-2 spike protein based on *in silico* analysis. This difference may allow a difference in the level of susceptibility of a person to SARS-CoV-2 infection (Calcagnile *et al.* 2021).

In addition to SNP, another phenomenon can affect the protein variability expressed from a gene, namely

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Alternative Splicing. This phenomenon strongly correlates with the expressed isoform variability contributing to phenotypic differences (Liu *et al.* 2017). For example, the Alternative Splicing phenomenon in the ACE2 gene may influence the infection of SARS-CoV-2 because it can produce different variants of the ACE2 isoform. However, studies about the effect of various variants due to the Alternative Splicing phenomenon on the ACE2 gene have not been widely reported, especially related to SARS-CoV-2 infection.

Knowledge of the Alternative Splicing phenomenon and the variants that can be produced could lead to innovative therapy treatments such as Splice-Switching Oligonucleotides (SSO), which can direct the splicing phenomenon to the desired variant (Havens & Hastings, 2016). Therefore, it is essential to know the splicing variant in the ACE2 gene that significantly affects SARS-CoV-2 infection.

This study aims to determine the effect of the Alternative Splicing phenomenon on the ACE2 gene on SARS-CoV-2 infection through the expressed ACE2 isoform based on *in silico* analysis.

MATERIALS AND METHODS

Sequence Search. The search for ACE2 isoform sequences was carried out through a search on the ensembl.org database (Yates *et al.* 2020). The investigation was carried out on protein options that have a Biotype Protein Coding. The protein sequence of the ACE2 isoform was obtained in .fasta format. The search for SARS-CoV-2 spike protein sequences was conducted by searching on the Protein Data Bank (PDB) www.rcsb.org (Burley *et al.* 2019). The SARS-CoV-2 spike protein sought was a consensus protein with the code 6XM0. The SARS-CoV-2 spike protein sequence was obtained in .pdb format.

Identity Percentage Analysis. Sequence similarity between the ACE2 isoforms and the ACE2 protein database was determined by Identity percentage analysis. This step was done to determine the modelling steps carried out by considering the results of the percentage of identities obtained (Waterhouse *et al.* 2018). This analysis was carried out using the https://blast.ncbi.nlm.nih.gov/Blast.cgi web server by selecting the blast protein option (Johnson *et al.* 2008).

3D Modeling and Validation of Model Results. 3D modeling of the ACE2 isoforms was carried out using a homology-based modeling approach through the Swiss Model with template 6M18.1.B (Waterhouse *et al.* 2018). After doing the 3D modeling, validated the model results using the Ramachandran plot approach (https://saves.mbi.ucla.edu/) and evaluated the z-score of the model obtained by comparing the z-score of protein structure obtained from the results of NMR and crystallography (https://prosa.services.came.sbg.ac.at/prosa.php) (Wiederstein & Sippl, 2007; Laskowski *et al.* 2013).

Protein Active Residue Prediction. The search for active residues of the ACE2 isoform and SARS-CoV-2 spike protein was carried out using CPORT, Consensus Prediction of Interface Residues in Transient Complexes (de Vries & Bonvin, 2011). After obtaining the predicted residues as active residues, a comparison was made with the literature. The selected active residue on the SARS-CoV-2 spike protein was a residue in the Receptor Binding Motif (RBM) region, which is the interaction area this protein possesses (Lan *et al.* 2020). Meanwhile, for the protein ACE2 isoform, residues in the peptidase domain were selected, which are the interaction domains of the SARS-CoV-2 spike protein on the ACE2 protein based on information from the ensembl.org database (Wang *et al.* 2020).

Molecular Docking and Docking Results Analysis. Molecular docking analysis was performed using the HADDOCK v.2.4 webserver https://wenmr.science.uu.nl/haddock2.4/submit/1 (van Zundert *et al.* 2016). The results obtained are then downloaded in .pdb format and the bond data is recorded. The bond data obtained were then analyzed for significance with each other by performing a one-way ANOVA test. Visualization of molecular docking results was performed by LigPlot+ v.1.4.5 and PyMOL Molecular Graphics System v.2.4.1, Schrödinger, LLC (Laskowski *et al.* 2011) to determine residues that play a role in visualized bonding.

RESULTS

This study obtained 11 different ACE2 transcript variants, each with its characteristics as shown in Table 1. The results of the BLAST protein performed for each variant with the protein-coding type showed a percentage of identity of more than 95% with the ACE2 protein (Accession no. BAB40370.1). Each ACE2 isoform sequence was then modeled with template 6M18.1.B and the validation results from each model obtained are shown in Table 1. The predicted results of the active residue on the ACE2 isoforms are also shown in Table 1 while the predicted active residue of the

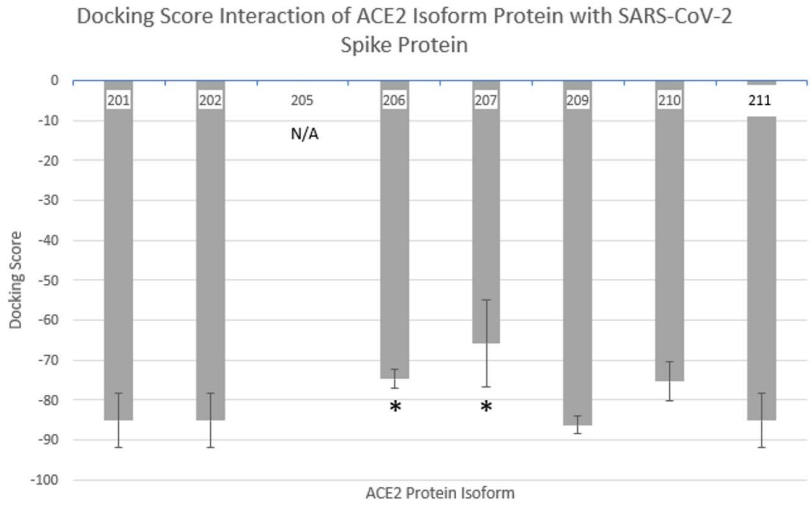

Fig 1 Docking score of the interaction of each protein variant of the ACE2 isoform against the SARS-CoV-2 spike protein obtained from the HADDOCK v.2.4 web server. The * sign indicates the significance based on the One-Way ANOVA test (α = 5%).

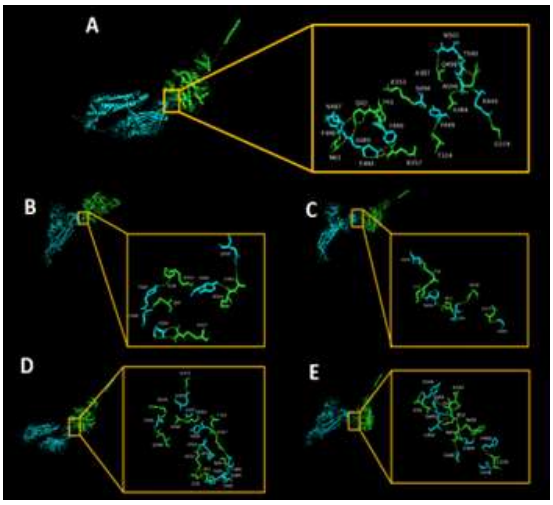

Fig 2 Visualization of interaction of SARS Cov-2 with ACE2_201, ACE2_202, ACE2_211 isoforms (A) ACE2_206 isoform (B) ACE2_207 isoform (C) ACE2_209 isoform (D) and ACE2_210 isoform.

SARS- CoV-2 are 449, 452, 453, 455, 470, 471, 474, 477, 478, 479, 480, 481, 482, 483, 484, 485, 486, 488, 489, 490, 493, 494, 496. Molecular Docking analysis between the SARS-CoV-2 spike protein and each ACE2 isoform is shown in Figure 1, with a visualization of the docking results shown in Figure 2.

DISCUSSION

The transcript variant used in this study, as shown in Table 1, is a transcript variant with a protein-coding type. The retained intron transcript and nonsense-mediated decay transcript variants were eliminated since it does not produce ACE2 isoforms (Yates *et al.* 2020). Therefore, the validation results of all models (Table 1) are considered to meet the criteria based on comparing the z-score and the favored region on the Ramachandran plot (Wiederstein & Sippl 2007; Laskowski *et al.* 2013).\

The predicted active residues in the ACE2 isoform (Table 1) showed F40, Y196, Y202, S218, W271, N338, W349, D350, H401 appeared in various isoform variants. However, in the ACE2_205 variant, no active residue was found. ACE2_205 variant has a short peptidase domain due to the splicing process on the exon encoding the domain. The peptidase domain itself is an integral part of the activity of the ACE2 protein, one of which is the binding site for the SARS-CoV-2 spike protein during infection (Wang *et al.* 2020; Shang *et al.* 2020). Therefore, the splicing process that removes many regions of the peptidase domain may eliminate its activity due to many essential residues that are not expressed.

Molecular docking results showed a significant

Table 1 The type of transcript, ID, model validation results, and the predicted results of the active residue of each ACE2 variant were obtained. N/A indicates that no data were obtained.

Variant	ID	Type of Transcript	Model Validation Result		Active Residue Prediction
			Value of Favoured Region in Plot Ramachandran	Z-Score	
ACE2_201	ENST00000252519.8	*Protein Coding*	92%	-11.73	F40, Y196, Y202, G205, E208, S218, R219, W271, A348, W349, D350, H401, D509
ACE2_202	ENST00000427411.2	*Protein Coding*	92%	-11.73	
ACE2_211	ENST00000680121.1	*Protein Coding*	92%	-11.73	
ACE2_203	ENST00000471548.5	*Nonsense Mediated Decay*	N/A	N/A	N/A
ACE2_204	ENST00000473851.1	*Retained Intron*	N/A	N/A	N/A
ACE2_205	ENST00000677282.1	*Protein Coding*	91.1%	-5.99	N/A
ACE2_206	ENST00000678046.1	*Protein Coding*	93.1%	-13.46	F40, S47, Y196, Y202, G205, S218, R219, W271, N338, C344, P346, T347, A348, W349, D350, H401, F504, N508, D509, Y510, S511, W606
ACE2_207	ENST00000678073.1	*Protein Coding*	92%	-11.73	N338
ACE2_208	ENST00000679162.1	*Nonsense Mediated Decay*	N/A	N/A	N/A
ACE2_209	ENST00000679212.1	*Protein Coding*	92%	-11.73	F40, Y50, T52, I54, T55, Y196, Y202, G205, E208, S218, W271, D335, P336, G337, N338, V339, Q340, K341, A342, V343, C344, A348, W349, D350, C361, H401
ACE2_210	ENST00000679278.1	*Protein Coding*	92%	-11.73	

difference in docking score between ACE2_206 and ACE2_207 variants against other variants except for ACE2_205 (Fig 2). Molecular Docking analysis cannot be performed on the ACE2_205 variant because there is no prediction of the active residue involved in the interaction. The differences in the docking score may indicate the interaction affinity of each variant of the ACE2 isoform with the SARS-CoV-2 spike protein (Calcagnile *et al.* 2021). Therefore, the protein variant ACE2_205 could not interact with the SARS-CoV-2 spike protein based on the results obtained, unlike the case with the ACE2_206 and ACE2_207 variants which can interact but have a lower binding affinity than the other variants. This difference in binding affinity seen from the difference in docking score may allow for differences in the level of vulnerability of a person who

has a specific variant as has been studied previously (Calcagnile *et al.* 2021; Chaudhary M., 2020; Devaux *et al.* 2020; Seyed *et al.* 2021; Suryamohan *et al.* 2021).

Based on the visualization results of the interaction of each protein variant of the ACE2 isoform with the SARS-CoV-2 spike protein (Figure 2), several key interaction residues appeared in each protein variant of the ACE2 isoform. Some residues such as Q42, N49, N53, E56, G319, K353, R357, M383, A384, and R559 appeared in several protein variants of the ACE2 isoform. Thus, the residues on the ACE2 isoform that appear in the interaction correspond to the interaction between ACE2 protein and SARS-CoV-2 spike protein observed by crystallography (PDB Code: 6CS2) (Kirchdoerfer *et al.* 2018). Therefore, this molecular docking result is acceptable.

This study showed differences in binding affinity to the SARS-CoV-2 spike protein, especially the isoform variants ACE2_205, ACE2_206, and ACE2_207 compared to others on molecular docking results. Specifically for the protein variant of the ACE2_205 isoform, no interactions were found with the SARS-CoV-2 spike protein. Therefore, this is an opportunity to develop COVID-19 therapy using SSO, which can direct the splicing variant to the protein variant of the ACE2_205 isoform so that there is the prevention of the interaction SARS-CoV-2 with target cells. One that should be considered in the development of SSO therapy is identifying regulatory elements that play a role in the Alternative Splicing process in the ACE2 gene to express the protein variant of the ACE2_205 isoform. The regulatory part referred to in this case is the cis-element in the mRNA transcript of the ACE2 gene (McManus & Graveley 2011). Therefore, specific oligonucleotides designed for SSO should consider the ability to attach to the cis-element side in the Alternative Splicing regulator in the ACE2 gene to influence the Alternative Splicing process.

Our findings can be used as a basis for reviewing the susceptibility of certain people to SARS-CoV-2 infection through the protein variant of the ACE2 isoform expressed in the body. Previous studies in the case of tauopathy show a neurodegenerative disease characterized by abnormalities in microtubule-associated protein (tau). This disorder involves an imbalance in the ratio of tau isoforms expressed through a single gene, MAPT (microtubule-associated protein tau). The imbalance of this ratio causes disturbances in the stability of microtubules in the axon to cause disorders in the nervous system (Sergeant *et al.* 2005). Alternative Splicing plays a vital role in regulating the balance of expression of various tau isoforms. Therefore, interference with Alternative Splicing itself can cause disturbances in the balance of tau isoform expression. Tauopathy disease detection can be done by analyzing variants of the resulting transcript to determine how the dynamics of isoform variations produced in the human body can cause the disease. Expression quantification is required to calculate the expression level of each transcript variant accurately. An unbalanced expression level can be used early in detecting this tauopathy (Jiang & Chen 2021). Therefore, detection of Tauopathy disease can be an idea in the development of detection of a person's susceptibility to SARS-CoV-2 infection through the expression ratio of the ACE2 gene transcript variant.

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