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INSILICO ANALYSIS OF CODON 131 POLYMORPHISM IN FCγRIIA GENE AND ASSOCIATION WITH CLINICAL SYMPTOMS PERSISTENCE OF DENGUE PATIENTS

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ABSTRACT

Background: Dengue Hemorrhagic Fever (DHF) is infection caused by Dengue Virus. Failure of vascularization is a main symptom of Dengue Hemorrhagic Fever inducing mediator secretion by an immune cell. FcγRIIA and CCL2 were a significant role in dengue pathogenesis and possibility factor for severe disease. **Objective:** Predictive bioinformatic analysis structure, function and expression of FcγRIIA mutant gene . **Methods:** Insilico analysis used NCBI database to find position and sequences. Analysis mutant use SNO and OMIM program. Protein prediction mutant gene use Uniprot program. **Result:** The accessed number of FcγRIIA human gene is NM_001136219. The length of gene was 2429 bp. had full name as Fc Fragment of IgG receptor IIa, located in 1q23.3 chromosom. analyzed mutation was rs1801274 with type of missense protein residue function experiencing a change from Histidin (H) turning into Arginin (R) with allele of wild-type A and becoming G amino acid position of 166**.** There was structural difference of FcγRIIA gene in wild type and mutant. The analysis. **Conclusion:** Insilico analysis Gene FcγRIIA mutant was a play a role of pathogenesis of dengue infection. Mutation in FcγRIIA had polymorfisme at Dengue Hemorragic Fever.
Keywords : *Dengue, FcγRIIA, Insilico, Mutant*

INTRODUCTION

FcR is a specific receptor of immunoglobulin (FcR). FcR had a significant role in immunity regulation. It was a trigger humoral-responsive immune resulting effector. Leucocyte receptor for IgG (*FcγR*), IgE (*FcεR*) and IgA (*FcαR*) were mediator in complex immune system. There was regulation of inflammation-cytokine secretion and host's resistant of the infection. *FcγRII* had some isoforms. There are b1, b2 and c. Majority distributed in the hemopoietic cell.[1] *FcγRIIA* is a family of Fc-receptor immunoglobin found in a surface of immune cell.[2]

Dengue infection is an infection caused by dengue virus (DENV), dominantly occurred in region of South Asia, Pacific and America.[3] DENV can result two types of infection such as primary and secondary.[4] During the secondary infection with different serotype, anti-body will form after the primary infection occurs.[5]

There are some types of Fcγ receptor, such as pre-dominant FcγRIIA (FcγR2a) expression in cell existing in the ADE mediation. It happens primarily in the interaction between anti-body antigen complex, and Fcγ receptor is a crucial component in the mechanism of immunologic effector variation, including phagocytosis, inflammation response, anti-body dependent cell mediated cytotoxicity and cleansing of immune complex.[6]

Failure of vascularization is a dominant symptom inducing mediator secretion by an immune cell.[7] SNP in the region promotor of the CCL2 gene in -2518 position influences the content of MCP-1.[8] Since FcγRIIA and CCL2 have a significant role in dengue pathogenesis and possibility in having a chance to cause dengue with a worse manifestation, it is beneficial to study allele variation of FcγRIIA to know the closely relatable growth of dengue infection and investigate characteristics of such gene.

Therefore, this paper presents an analysis of bio-informatic structure, function and expression of FcγRIIA mutant gene. It showed an analysis of genetic mutation of FcγRIIA and protein sequence, and is compared between wild-type sequence and mutant. In addition, the article performs 3D-structure analysis.

METHOD

Selection of DNA sequences and FcγRIIA Protein

The sequence was obtained from the database of National Center for Biotechnology Information (NCBI), retrieved from https://www.ncbi.nlm.nih.gov/gene It was selected based on a completeness of protein and nucleotide

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accession, Single Nucleotide Polymorphism (SNP) and a sequence of amino acid (AA). The selected SNP data was based on the study of Dettogni, RS et al. in 2015, entitled Single nucleotide polymorphisms in immune system genes and their association with clinical symptoms persistence in dengue infected persons, published in the journal of American Society for Histocompability and Immunogenetics.[9]

A selection process resulted 1 complete sequence of *FcγRIIA* human with access number of NM_001136219.1, consisting of 2429 bp, which is expressively Fc fragment of IgG receptor IIa homo sapiens.

Analysis of mutation

This analysis was performed via NCBI utilizing SNP and OMIM menu. In the SNP page, table was shown describing characteristic of mutating *FcγRIIA* gene. Hence, an analyzed mutation was rs1801274 with type of missense protein residue function experiencing a change from Histidin (H) turning into Arginin (R) with allele of wild-type A and becoming G amino acid position of 166.[10]

Prediction Of Peptide Signal And Trans-Membrane Protein

Analysis of the sequence of *FcγRIIA* amino acid protein

The analysis of the sequence of amino acid in FCGR2A protein was conducted by identifying existing characteristic in such protein. In detail, it analyzed the existence of peptide signal, trans-membrane, and topology of such protein. Those used some different online sites.

3D-structure analysis of wild-type and mutant from *FcγRIIA* protein

3D-structure in either wildtype dan mutant of FCGR2A gene was predicted using the pymol program. The protein was compared based on composition and protein structure resulted and based on the relation with surrounding acid amino. The comparison of binding site from *FcγRIIA* gene was via https://prosite.expasy.org/

RESULT

Characteristic of *FcγRIIA*

FCGR2A with access number of NC_000001.1 is a gene coding one of families from immunoglobulin of Fc gene receptor, found in the surface of immune cell. A coded protein by such cell is a surface receptor existing in the macrophage cell, such as macrophage, neutrophil, having contribution in immunity process. *FcγRIIA* human with access number of NM_001136219 by a length of 2429 bp has its full name as Fc Fragment of IgG receptor IIa, located in 1q23.3 chromosome, by total of exon of 11. (Figure 1).

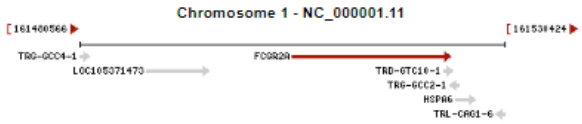

Figure 1. Position *FcγRIIA* gen on first chromosome https://www.ncbi.nlm.nih.gov/gene

Mutant analysis with OMIM and SNP

A mutation occurred in *FcγRIIA* gene is based on SNP analysis with the code of rs1801274, in accordance with previous journal. It is in the codon of 166, with type of missense mutation and change of amino acid of H (histidine) becoming R (arginin) H/R. The sequence is as attached.

According to codon analysis experiencing mutation via OMIM (omim.org), it is found that mutation of FCGR2A gene with chromosome area of 1q23.3 results on phenotype deficiency, as follows: (https://omim.org/entry/146790)

1. Lupus nephritis susceptibility to
2. Malaria, severe, susceptibility to
3. Pseudomonas Aeruginosa susceptibility to chronic infection by Incystic fibrosis

Prediction of Peptide Signal

Peptide signal is a short peptide in the part of N-terminal protein, carrying information of protein secretion course. It is in both prokaryotic dan eukaryotic. Typically, the length of peptide signal is around 25-35 residue. However, a long peptide signal can reach 140 residue, usually found not only in eukaryotic, but also in virus protein and auto-transport bacteria.[12] Further, peptide signal is predicted using software derived from www.cbs.dtu.dk, and the result of prediction is produced as follows.

Based on figure 2, it depicts that peptide signal of FCGR2A gene was due to C, Y, S value that was above cut-off value as of 0,450.

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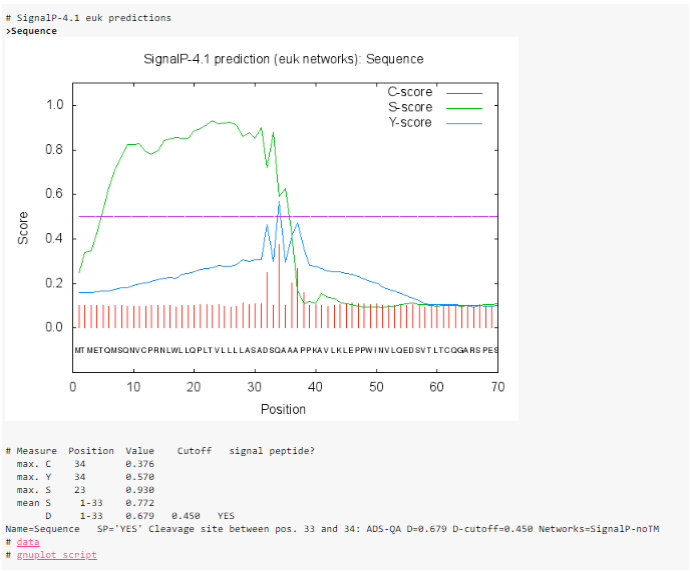


Figure 2. Result of Peptide Signal on FCGR2A Gene Using www.cbs.dtu.dk

Prediction of Trans-membrane Protein

Trans-membrane protein is found in membrane of all cells and cellular organelle. Such protein is a highly important for cells functioning as normal as possible. For example, much naturally occurring trans-membrane protein is the movement line of specific substance crossing such membrane. Ample of trans-membrane protein receives and sends such signal.

This prediction uses online software of TMHMM. TMHMM is a method to predict helix based-trans-membrane protein, according to Markov model and developed by Anders Krogh and Erik Sonnhammer. The official website employed is http://www.cbs.dtu.dk/services/ TMHMM/ and FCGR2A gene is obtained.

Table 1. Topology of Transmembran Protein FCGR2A human (TMHMM)

Protein Sequence (AA)		***Orientation***
From	***To***	
1	217	*Outside*
218	240	*Tmhelix*
241	317	*Inside*

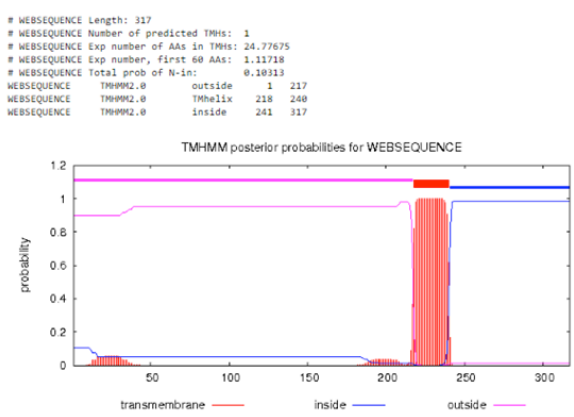
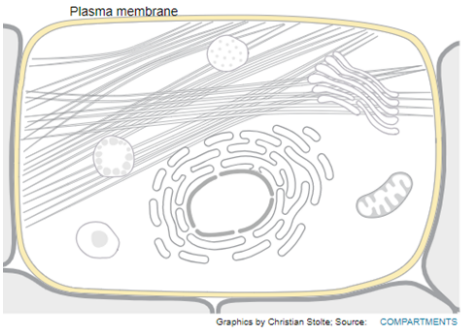


Figure 3. Analisis result transmembran protein *FcγRIIA* gene http://www.cbs.dtu.dk/services/TMHMM/

From above finding, it shows that FCGR2A gene had trans-membrane protein, whose length was 317 and total of prediction was 1. Based on the produced sequence, it was found in position of 1-121, Tm helix position of 218-240 and inside position of 241-317.

Protein prediction with Uniprot

Protein prediction was conducted to know the function and location of sub-cellular, and membrane plasma employing www.uniprot.org. The function of *FcγRIIA* gene is a binding with Fc region in the part of IgG, which is a receptor with low affinity bound on IgG initiating cellular response to pathogen and soluble anti-gene. Also, it functions to perform anti-gene phagocytosis promotion with opsonization. Based on the location of gene's sub-cellular, it is in membrane plasma.[13]



*Existing position in cell's membrane = broken-white in color

Figure 4. Location analysis of *FcγRIIA* gene cellular employing www.uniprot.org

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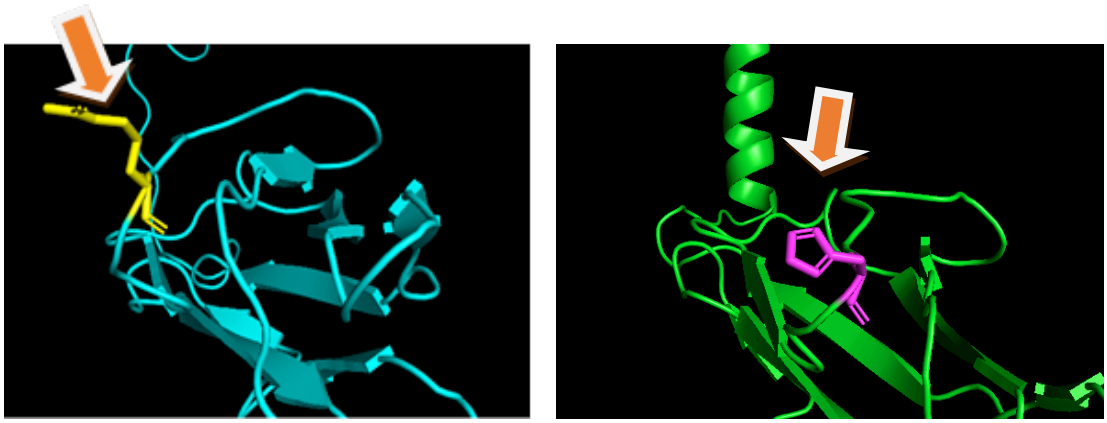
Structure prediction of *FcγRIIA*

Structure prediction of wild type protein and mutant was performed by inputting their sequence, such as acid amino in the web of swissmodel. Later, it was analyzed using pymol. The analysis was performed by viewing structure and formation change of the protein prediction. Particularly, the function of a protein depends on structure possessed by such protein depending on physics' and chemical's parameter structure. It is additionally valuable to scientifically know molecule's nature biologically, physically, chemically, mathematically, and informatically, so that the form of its cooperation is known.[14]



(a) (b)

Figure 5. Prediction structure wildtype (a) and Mutan (b) with spot mutation *FcγRIIA* gene

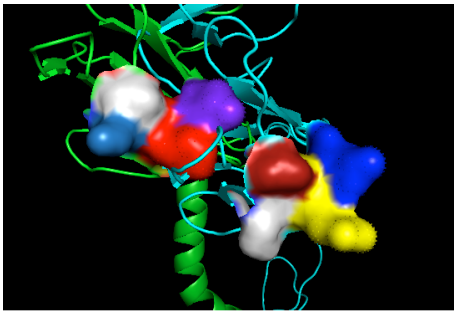


Figure 6. Structure prediction wildtype and mutan *FcγRIIA* gene

Figure 7. Alignment Wildtype dan Mutan *FcγRIIA* gene

Figure 5 displays that there was structural difference of *FcγRIIA* gene in wild type and mutant. It was seen from the structure of acid amino from both, depicting different form. Figure 6 demonstrated 3D analysis to observe whether or not there was interaction changing in a protein caused by the existence of mutation of *FcγRIIA* gene. Further, the form of primary structure resulted by such mutation has shown a different result between wild type and mutant, so the function changing possibly occurs in *FcγRIIA* gene mutant.

Table 2. Interaction amino acid (AA) with mutan amino acid in the sequence at 166

AA number	AA name
151	Valine (V)
152	Lysine (K)
165	Serine (S)
167	Leusin (L)

Analysis with acid amino surrounding mutation point describes that there was interaction changing with surrounding acid amino. In detail, there were 4 acid amino having direct contact with mutation point, so a structural difference from such

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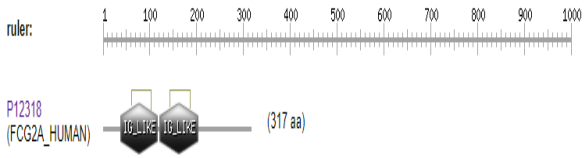
mutation occurred. Presumably, acid amino having deformation was due to interaction with acid amino resulted from mutation in the sequence of 151, 152, 165 and 167.

Binding Site Protein *FcγRIIA*

Protein *FcγRIIA* protein have two binding site based *prosite expasy*. Binding site is in position 39-118 and 122-204. *FcγRIIA* mutan in sequence 166 so have in second binding site. This is can effect to phagocytosis which is monocyte cell function.



RecName: Full=Low affinity immunoglobulin gamma Fc region receptor II-a; Short=IgG Fc receptor II-a; AltName: Full=CDw32; AltName: Full=Fc-gamma RII-a; Short=Fc-gamma-RIIa; Short=FcRII-a; AltName: CD_antigen=CD32; Flags: Precursor;. *Homo sapiens (Human)* https://prosite.expasy.org/cgi-bin/prosite/ScanView

Figure 8. Binding site position in *FcγRIIA* gene

Table 3. Comparison protein composition gene wildtype dan mutan

Item	FcγRIIA			
	Wildtype		Mutan	
Number of amino acids*	286		317	
Molecular weight*	31477,74		35019,73	
Theoretical PI*	6,18		6,3	
Asam amino composition	Ala (A)*	7 %	Ala (A)	7,3 %
	Arg (R)*	2,4 %	Arg (R)	3,5 %
	Asn (N)	6,3 %	Asn (N)	6,3 %
	Asp (D)*	4,5 %	Asp (D)	4,7 %
	Cys (C)*	2,1 %	Cys (C)	1,9 %
	Gln (Q)*	5,6 %	Gln (Q)	6,0 %
	Glu (E)*	3,5 %	Glu (E)	4,1 %
	Gly (G)*	4,5 %	Gly (G)	4,4 %
	His (H)*	3,5 %	His (H)	2,8 %
	Ile (L)*	4,9 %	Ile (L)	5,0 %
	Leu (L)*	9,4 %	Leu (L)	8,8 %
	Lys (K)*	4,2 %	Lys (K)	4,4 %
	Met (M)*	2,4 %	Met (M)	2,5 %
	Phe (F)	2,8 %	Phe (F)	2,8 %
	Pro (P)*	7,3 %	Pro (P)	7,6 %
	Ser (S)*	9,1 %	Ser (S)	8,5 %
	Thr (T)*	8,4 %	Thr (T)	8,2 %
	Trp (W)*	1,7 %	Trp (W)	1,6 %
	Tyr (Y)*	2,8 %	Tyr (Y)	2,5 %
	Val (V)*	7,3 %	Val (V)	6,9 %
	Pyl (O)	0,0 %	Pyl (O)	0,0 %
	Sec (U)	0,0 %	Sec (U)	0,0 %
Total number of negatively charged residues (Asp + Glu)*	23		28	
Total number of positively charged residues (Asp + Glu)*	19		25	
Atomic composition :*				
Carbon	1396		1546	
Hydrogen	2182		2432	
Nitrogen	378		426	
Oxygen	425		474	
Sulfur	13		14	
Formula*	$C_{1396}H_{2182}N_{378}O_{425S13}$		$C_{1546}H_{2432}N_{426}O_{474}S_{14}$	
Total number atom*	4394		4892	
Aliphatic index*	84,20		81,51	

*have different composition https://web.expasy.org/cgi-bin/protparam/protparam

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DISCUSSION

Many study have revealed that any mutation of *FcγRIIA* had polymorphism related to Dengue Fever or Dengue Hemorrhagic Fever. Study in Kuba, a patient infected by DENV-4 and there was *FcγRIIA* polymorphism has reported that allele R and RR were closely related to a protection to symptomatic dengue; while, allele H and HH genotype were linked to dengue fever and dengue hemorrhagic fever. Another research performed in Vietnam describes that a case of dengue hemorrhagic fever suffered by children revealed a significant relation of allele R genotype and RR from *FcγRIIA*, producing a protection to DHF. However, other researches conducted in Mexico display that HH genotype was related to more minor and vulnerable infection, rarely turning to severe case. In addition, some researches state that there was a distribution of specific population showing variation of allele and research design. [15]

Some study describe that mutation in *FcγRIIA* have polymorfisme at Dengue Hemorragie Fever. A study in Kuba describe that patient which is DENV-4 infection have *FcγRIIA* polymorfism. Alel R and RR have role to dengue simptomatic protection, while alel H and HH have role dengue fever and dengue hemorrhage fever. Another study from Vietnam describe that dengue fever in children have significant relationship with R and RR alel genotipe *FcγRIIA* gene which is DHF protect function. But, study in Mexico describe that HH genotipe related with mild infection and less in severe case DHF. Several studies state that there are differences in the distribution of specific populations that show allele variations and research designs.[15]

The study in Pakistan reinforces the evidence for the FcγRIIa polymorphisme as an inherited genetic determinant in clinical outcome dengue infection. The possible mechanism behind this association of FcγRIIa mutant polymorphism with clinical outcome in dengue disease can be explained, at least partially, in the perspective of ADE theory. Previously, it has been shown that the IgG1 and IgG3 are the prime immunoglobulins produced during the course of dengue infection and FcγRIIa serves as a commonly distributed receptor for all IgG subclasses.[16]

CONCLUSION

Insilico analysis *FcγRIIA* mutant gene is still a play a role of pathogenesis of dengue infection. Mutation in *FcγRIIA* have polymorfisme at Dengue Hemorrage Fever.

CONFLICT OF INTEREST

No conflict of interest in this study

REFERENCES

1. Powell MS, Barton PA, Emmanouilidis D, Wines BD, Neumann GM. Biochemical analysis and crystallisation of Fc g RIIa , the low affinity receptor for IgG. 1999;68:17–23.
2. NCBI. FCGR2A Fc fragment of IgG receptor IIa [Homo sapiens (human)] [Internet]. Gene. 2018.
3. Guzman MG, Harris E. Dengue. Lancet [Internet]. 2015;385(9966):453–65.
4. Journal CM, Journal CM. Shift in the transmission pattern of dengue serotypes and concurrent infection with more than one dengue virus serotype. 2011;176–8.
5. Guzman MG, Vazquez S. The Complexity of Antibody-Dependent Enhancement of Dengue Virus Infection. 2010;2649–62.
6. Moi ML, Lim C, Takasaki T, Kurane I. Involvement of the Fc c receptor IIA cytoplasmic domain in antibody-dependent enhancement of dengue virus infection. 2018;(2010):103–11.
7. Herrero LJ, Zakhary A, Gahan ME, Nelson MA, Herring BL, Hapel AJ, et al. Pharmacology & Therapeutics Dengue virus therapeutic intervention strategies based on viral , vector and host factors involved in disease pathogenesis. Pharmacol Ther. 2013;137(2):266–82.
8. Sierra B, Perez AB, Vogt K, Garcia G, Schmolke K, Aguirre E, et al. Cytokine MCP-1 and MIP-1 a expression in a model resembling early immune response to dengue. Cytokine. 2010;52(3):175–83.
9. Spinassé R, Tristão-sá R, Figueiredo F. Single nucleotide polymorphisms in immune system genes and their association with clinical symptoms persistence in dengue-infected persons. 2015;76:717–23.

DIPONEGORO MEDICAL JOURNAL
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Volume 10, Number 6, November 2021

10. NCBI. Reference SnP Cluster Report : rs1801274 [Internet]. 2018.
11. NCBI. Fc FRAGMENT OF IgG, LOW AFFINITY IIa, RECEPTOR FOR; FCGR2A [Internet].
12. Owji H, Nezafat N, Negahdaripour M, Hajiebrahimi A. European Journal of Cell Biology A comprehensive review of signal peptides : Structure , roles , and applications. Eur J Cell Biol. 2018;97(6):422–41.
13. UniprotKB-P12318 [Internet].
14. Luscombe NM1, Greenbaum D GM. What is Bioinformatics? A proposed definition and overview of the field. methods Inf Med. 2001;40(4):346–58.
15. Alagarasu K, Bachal R V, Damle I, Shah PS, Cecilia D. Association of FCGR2A p . R131H and CCL2 c . -2518 A > G gene variants with thrombocytopenia in patients with dengue virus infection. Hum Immunol. 2015;76(11):819–22.
16. Saima Naz Mohsin, Saqib Mahmood, Ali Amar et al. Association of FCγRIIA Polymorphism with clinical outcome of dengue infection : First Insight from Pakistan. Am J Trop Med Hyg. 2015; 93(4):691-696.