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 mutation 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 chromosomal 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 polymorphism at Dengue Hemorrhagic Fever. Keywords: Dengue, FcγRIIA, Insilico, Mutant

INTRODUCTION

Fcγ is a specific receptor of immunoglobulin (FcR). FcR has a significant role in immunity regulation. It was a trigger humoral-responsive immune resulting effector. Leucocyte receptor for IgG (FcγR), IgE (FceR) and IgA (FcaR) 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. FcγRIIA is a family of Fc-receptor immunoglobulin 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
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 Histocompatibility and Immunogenetics.  

A selection process resulted in 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.  

**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, transmembrane, 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 gene 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. 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.
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)

<table>
<thead>
<tr>
<th>Protein Sequence (AA)</th>
<th>Orientation</th>
</tr>
</thead>
<tbody>
<tr>
<td>From 1</td>
<td>217</td>
</tr>
<tr>
<td>218</td>
<td>240</td>
</tr>
<tr>
<td>241</td>
<td>317</td>
</tr>
</tbody>
</table>

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

Figure 3. Analisis result transmembran protein FcyRIIA 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 FcyRIIA 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

Figure 4. Location analysis of FcyRIIA gene cellular employing www.uniprot.org
Structure prediction of $Fc_yRIIA$

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

![Figure 5. Prediction structure wildtype (a) and Mutan (b) with spot mutation $Fc_yRIIA$ gene](image)

Figure 5 displays that there was structural difference of $Fc_yRIIA$ 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 $Fc_yRIIA$ 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 $Fc_yRIIA$ gene mutant.

![Figure 6. Structure prediction wildtype and mutan $Fc_yRIIA$ gene](image)

![Figure 7. Alignment Wildtype dan Mutan $Fc_yRIIA$ gene](image)

<table>
<thead>
<tr>
<th>AA number</th>
<th>AA name</th>
</tr>
</thead>
<tbody>
<tr>
<td>151</td>
<td>Valine (V)</td>
</tr>
<tr>
<td>152</td>
<td>Lysine (K)</td>
</tr>
<tr>
<td>165</td>
<td>Serine (S)</td>
</tr>
<tr>
<td>167</td>
<td>Leusin (L)</td>
</tr>
</tbody>
</table>

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

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
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.

<table>
<thead>
<tr>
<th>Item</th>
<th>Wildtype</th>
<th>Mutan</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of amino acids*</td>
<td>286</td>
<td>317</td>
</tr>
<tr>
<td>Molecular weight*</td>
<td>31477,74</td>
<td>35019,73</td>
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<tr>
<td>Theoretical PI*</td>
<td>6,18</td>
<td>6,3</td>
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<tr>
<td>Ala (A)*</td>
<td>7 %</td>
<td>Ala (A)</td>
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<tr>
<td>Arg (R)*</td>
<td>2,4 %</td>
<td>Arg (R)</td>
</tr>
<tr>
<td>Asn (N)</td>
<td>6,3 %</td>
<td>Asn (N)</td>
</tr>
<tr>
<td>Asp (D)*</td>
<td>4,5 %</td>
<td>Asp (D)</td>
</tr>
<tr>
<td>Cys (C)*</td>
<td>2,1 %</td>
<td>Cys (C)</td>
</tr>
<tr>
<td>Gln (Q)*</td>
<td>5,6 %</td>
<td>Gln (Q)</td>
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<tr>
<td>Glu (E)*</td>
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</tr>
<tr>
<td>Gly (G)*</td>
<td>4,5 %</td>
<td>Gly (G)</td>
</tr>
<tr>
<td>His (H)*</td>
<td>3,5 %</td>
<td>His (H)</td>
</tr>
<tr>
<td>Ile (L)*</td>
<td>4,9 %</td>
<td>Ile (L)</td>
</tr>
<tr>
<td>Leu (L)*</td>
<td>9,4 %</td>
<td>Leu (L)</td>
</tr>
<tr>
<td>Lys (K)*</td>
<td>4,2 %</td>
<td>Lys (K)</td>
</tr>
<tr>
<td>Met (M)*</td>
<td>2,4 %</td>
<td>Met (M)</td>
</tr>
<tr>
<td>Phe (F)</td>
<td>2,8 %</td>
<td>Phe (F)</td>
</tr>
<tr>
<td>Pro (P)*</td>
<td>7,3 %</td>
<td>Pro (P)</td>
</tr>
<tr>
<td>Ser (S)*</td>
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<td>Ser (S)</td>
</tr>
<tr>
<td>Thr (T)*</td>
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<td>Thr (T)</td>
</tr>
<tr>
<td>Trp (W)*</td>
<td>1,7 %</td>
<td>Trp (W)</td>
</tr>
<tr>
<td>Tyr (Y)*</td>
<td>2,8 %</td>
<td>Tyr (Y)</td>
</tr>
<tr>
<td>Val (V)*</td>
<td>7,3 %</td>
<td>Val (V)</td>
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<td>Pyl (O)</td>
<td>0,0 %</td>
<td>Pyl (O)</td>
</tr>
<tr>
<td>Sec (U)</td>
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<td>Sec (U)</td>
</tr>
<tr>
<td>Total number of negatively charged residues (Asp + Glu)*</td>
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<td>28</td>
</tr>
<tr>
<td>Total number of positively charged residues (Asp + Glu)*</td>
<td>19</td>
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<td>Atomic composition:*</td>
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<tr>
<td>Carbon</td>
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<td>1546</td>
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<tr>
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<td>2182</td>
<td>2432</td>
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<tr>
<td>Nitrogen</td>
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<td>426</td>
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<tr>
<td>Oxygen</td>
<td>425</td>
<td>474</td>
</tr>
<tr>
<td>Sulfur</td>
<td>13</td>
<td>14</td>
</tr>
<tr>
<td>Formula*</td>
<td>C1396H2182N378O425S13</td>
<td>C1546H2432N426O474S14</td>
</tr>
<tr>
<td>Total number atom*</td>
<td>4394</td>
<td>4892</td>
</tr>
<tr>
<td>Aliphatic index*</td>
<td>84,20</td>
<td>81,51</td>
</tr>
</tbody>
</table>

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

Some study describe that mutation in FcγRIIA have polymorphism at Dengue Hemorrhagic Fever. A study in Kuba describe that patient which is DENV-4 infection have FcγRIIA polymorphism. Allele R and RR have role to dengue simptomatic protection, while allele 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 allele 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. 

The study in Pakistan reinforces the evidence for the FcγRIIa polymorphism 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.

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

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