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Short Communications

DC-SIGN (CD209) Carbohydrate Recognition Domain Is Not Polymorphic in Dengue Virus-Infected Indonesian Patients

Tri Wibawa[1]*, Nastiti Wijayanti[2], Eggi Arguni[3] and Ida Safitri Laksono[3]

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Abstract: Dengue virus (DENV) infection is a significant burden in Indonesia and other tropical countries. DENV infection has a wide spectrum of clinical manifestations, i.e. asymptomatic, dengue fever, dengue hemorrhagic fever and dengue shock syndrome. The variety of clinical manifestations may be due to the diversity of genetic constitution of the host. The C-type lectin DC-SIGN (CD209) has been identified as the major dengue receptor on human dendritic cells. There are at least five polymorphisms in exon 5 and 6 of the DC-SIGN encoded gene which have been identified and recorded in dbSNP. The aim of this work is to measure the frequency of these polymorphisms among asymptomatic and hospitalized DENV-infected patients. We enrolled 23 hospitalized and 73 asymptomatic DENV-infected patients. Among the subjects, we performed PCR amplification and DNA direct seqencing for 23 hospitalized DENV-infected patients and 24 asymptomatic DENV-infected patients. The result showed that there were no polymorphic nucleotides in the CD209 encoded gene among the patients.
Key words: DC-SIGN, dengue virus infection, polymorphism

Introduction

Dengue virus (DENV) infection is a significant burden in Indonesia and other tropical countries. The magnitude of incidence and prevalence are increasing. For more than 40 years, countries endemic for DENV infection have reported hospitalized cases and deaths. For the period 1956–2004, there were 4,975,807 dengue haemorrhagic fever (DHF) cases (a small proportion of these are dengue fever) with 68,977 deaths. The case fatality rate was around 1.4%. DHF is a huge problem in Southeast Asia. The deaths due to DENV infection average 1,682 per year [1, 2].

DENV infection has a wide spectrum of clinical manifestations, i.e. asymptomatic, dengue fever (DF), dengue haemorrhagic fever (DHF) and dengue shock syndrome (DSS). Asymptomatic and dengue fever patients usually do not need hospitalization. However, patients suffering from DHF and DSS need intensive medical attention. These syndromes are caused by any of the four DENV serotypes (DENV1, DENV2, DENV3, and DENV4) that belong to the family Flaviviridae [3]. The incubation period for DENV infection is 4–6 days, before clinical manifestation are observed. The chief complaint of patients is usually fever, and some cases show maculopapular rash. Other patients may show classical symptoms of DENV infection, such as fever, joint pain, headache, muscle pain and rash. After 3–4 days from onset, the fever usually improves. More severe patients may present bleeding manifestations, decreased platelet count, and hemoconcentration. Fluid and electrolytes are the recommended therapy for DENV infection cases. A small portion of these cases (5–10%) may be fatal because of shock. Plasma leakage is the main characteristic of severe DENV infection [3].

The determinant factors that influence clinical manifestations and outcomes are not well understood. There are myriad interactions of viral virulence, immunological processes, and factors related to human genetics. The influence of human genetic factors on DENV infection was revealed by studies comparing ethnic groups, blood type and human leukocyte antigen (HLA) polymorphisms [4]. Later on, many studies analyzed the association of the polymorphisms of certain human genes with susceptibility to DENV infection and clinical outcomes. Several genes

[1] Department of Microbiology, Faculty of Medicine, Universitas Gadjah Mada, Indonesia
[2] Laboratory of Animal Phisiology, Faculty of Biology, Universitas Gadjah Mada, Indonesia
[3] Department of Pediatrics, Faculty of Medicine, Universitas Gadjah Mada / Dr. Sardjito General Hospital, Indonesia
*Corresponding author:
Department of Microbiology, Faculty of Medicine, Universitas Gadjah Mada, Jl. Farmako, Sekip Utara, Yogyakarta 55281 Indonesia
Tel: +62274580297
Fax: +62274581876
E-mail: twibawa@ugm.ac.id

were studied with various results i.e *CD32*, vitamin D receptor (*VDR*), interleukin 1 receptor (*IL-1RA*), *IL-4*, manose binding lectin (*MBL2*), *TNFA*, *IL-10*, transforming growth factors (*TGFB1*), interferon gama (*IFNG*), *IL-6*, *DC-SIGN* (CD209), *TAP1*, and human platelet alloantigens (*PAI*). Among the genes studied, *VDR*, *TNFA*, *DC-SIGN* promoter region, and *TAP1* are significantly associated with DENV infection [5]. The C-type lectin DC-SIGN (CD209) has been identified as the major DENV receptor on human dendritic cells [6]. The role of DC-SIGN (CD209) in DENV infection is important since a higher DENV concentration in the blood has been shown to be significantly correlated with disease severity [7]. A single nucleotide polymorphism (SNP) in the promoter region of CD209 (2336 A/G; rs4804803) is associated with host susceptibility to many infectious diseases, such as DENV infection [8–11], human immuno-deficiency virus 1 infection [12] and tuberculosis [13].

DC-SIGN is a tetrameric molecule, which acts as a receptor in the dendritic cell and mediates cell-cell interaction. One of the important extracellular domains of this calcium-dependent C-type lectin family is a carbohydrate recognition domain (CRD), which interacts calcium-dependently with various pathogens. The other two domains are a cytoplasmic and transmembrane domain [6]. DC-SIGN is encoded by a gene located in the chromosome 19p13. CRD corresponds to exon 5–7 of DC-SIGN encoded gene.

At least five polymorphisms in exon 5 and 6 of DC-SIGN encoded gene have been identified and recorded in dbSNP (http://www.ncbi.nlm.nih.gov/snp/), namely: rs138694095 (GAT→GGT), rs11465393 (GCC→TCC); rs148485547 (TCA→TAA); rs146002807 (ACG→ATG); and rs75008691 (CGC→CAC). These SNPs were located close to each others which makes amplification with one pair primer possible.

The accumulated data showed that the CRD of the DC-SIGN is important for interaction with the pathogens. Since there are many SNPs located in this domain, it is interesting to study the association of SNPs located in the CRD of DC-SIGN with the DENV infection susceptibility of the Indonesian population. We measured the frequency of these polymorphisms among asymptomatic and hospitalized DENV infection patients. Our data showed that there was no polymorphic area within the CRD of the DC-SIGN that may contribute to the genetic susceptibility of the patients to DENV infection.

Methods

a. Subjects

Hospitalized Group

Subjects in the hospitalized group were enrolled according to the following inclusion criteria: patients diagnosed with DENV infection, confirmed by either NS1 or RT-PCR test. Patients were recruited by two schemes: either using archive blood samples collected from previous studies or actively recruiting new patients from the Dr. Sardjito General Hospital. NS1 or RT-PCR test were done to confirm DENV infection. Informed consent was obtained from patients before collecting 3-ml samples of blood for the subsequent polymorphisms analysis. There were 23 subjects enrolled in this group.

Asymptomatic Group

The controls were in-house IgM-positive subjects enrolled in a previous study (unpublished). The healthy subjects were recruited with new informed consent. During home visits, subjects were recruited and asked to donate 3-ml blood samples for this study. There were 73 subjects enrolled in this group, which were matched with the following inclusion criteria: IgM positive and had no history of fever within three months before enrolment. The medical and health research ethics committee of the Faculty of Medicine Universitas Gadjah Mada / Dr. Sardjito General Hospital granted ethical approval for this work.

DNA Extraction

DNA were extracted from whole blood using GeneJet Genomic DNA Purification Kit (Fermentas) according to the manufacturer's suggested protocol.

PCR Amplification

PCR amplification was done using Dream Taq Green Mastermix kit (Fermentas) according to the manufacturer's suggested protocol. Primers were designed according to the full sequence of Homo sapiens CD209 molecule (CD209), transcript variant 1, mRNA (NCBI Reference Sequence: NM_021155.3). The primer sequences are as follows: CRD-2F: 5' AAC TTC CTA CAG CTG CAG TC 3' and CRD-1R: 5' TGG AGA GAA GGA ACT GTA GC 3'. PCR amplification produced 1218 bp amplicon. The primers were designed so as to correspond to the flanking area of the five reported SNPs in exon 5 and 6 of of CD209 encoded gene.

DNA Sequencing

The SNPs were detected by the direct sequencing method. PCR amplification products were purified and se-

quenced. Each sample was sequenced using forward primer and confirmed using reverse primer.

Results and Discussion

There were 23 DENV-infected patients in the hospitalized group, and 73 subjects in the asymptomatic group were enrolled. Blood samples were collected and DNA was extracted. PCR amplification succeeded in 23 subjects (13 male and 10 female) in the hospitalized group and 24 subjects (12 male and 12 female) in the asymptomatic group (Fig. 1). The PCR products were subsequently subjected to DNA sequencing.

The regions corresponding to the locus previously reported as polymorphic were: rs138694095 (GAT→GGT), rs11465393 (GCC→TCC); rs148485547 (TCA→TAA); rs146002807 (ACG→ATG); and rs75008691 (CGC→CAC), were sequenced (Fig. 2). The results showed that there was no polymophism among the two groups.

We expected to find polymorphism in the carbohy-

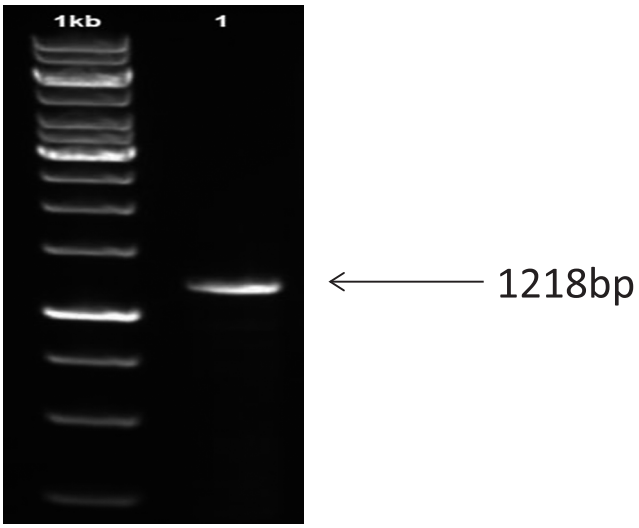

Fig. 1. Electrophoresis of representative purified – PCR product pro DNA Sequencing. PCR amplification were done using primer CRD-2F and CRD-1R which correspond to the sequence of genomic DNA exon 5 and 6 of CD209 encoded gene.

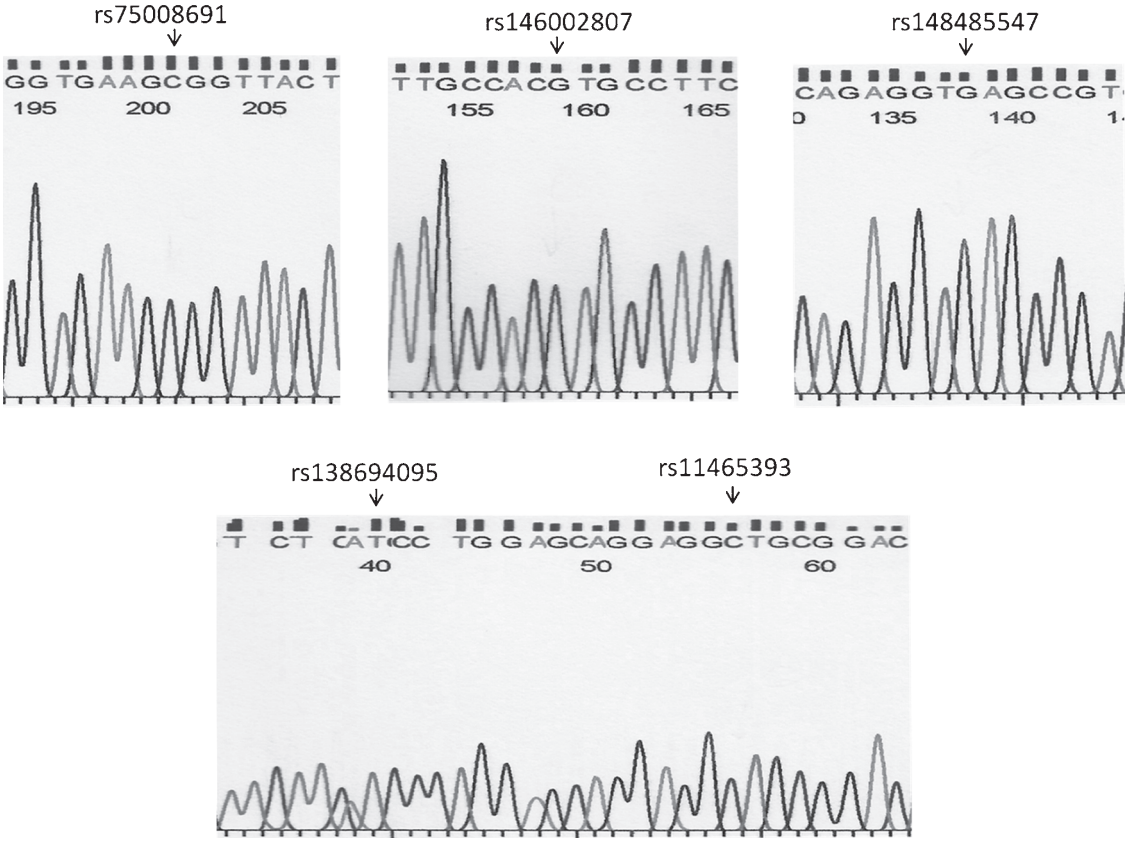

Fig. 2. DNA Sequencing of DC-SIGN (CD209) carbohydrate recognition domain (CRD) exon 5 and 6. Arrows denote the location of SNPs previously reported in the dbSNPs database: namely: rs138694095 (GAT→GGT), rs11465393 (GCC→TCC); rs148485547 (TCA→TAA); rs146002807 (ACG→ATG); and rs75008691 (CGC→CAC). The SNPs were not identified in Indonesian DENV-infected patients.

drate recognition domain (CRD) of *DC-SIGN* that may be associated with the genetic susceptibility to DENV among Indonesian patients, but the results showed that the CRD of *DC-SIGN* in Indonesian is not polymorphic, providing another example that polymorphism is an ethnically and geographically dependent variable. The polymorphism reported in certain ethnic and geographical areas may not be found in another areas. It may be also related to the different precipitating mechanisms of diseases [14].

There are several factors that may govern the susceptibility of patients to DENV infection. One approach is to identify the genetic diversity related with the candidate receptors for the entry of DENV into the human cells. However, there are several candidate DENV receptors in human cells. Carbohydrate molecules such as sulfated glycosaminoglycans (GAGs) and glycosphingolipid (GSL) are expected to act as co-receptor molecules to facilitate the virus entry. Lectins expressed on dendritic cells (DCs) and macrophages under the human skin are involved in the initial contact of DENV introduced by mosquito bites. DC-SIGN is the most characterized lectin regarding its function in DENV entry. Factors related to protein folding, such as heat shock proteins and chaperones, were reported to be essential for DENV2 entry into the host cells. High-affinity laminin receptor and CD14-associated protein may also regulate DENV-host cell interaction [15].

A SNP in the promoter region of CD209 (2336 A/G; rs4804803) was associated with host susceptibility to many infectious diseases, including DENV infection [8–11]. Polymorphism in the receptor region may result in decreased CD209 expression. This may lead to the attachment of DENV in the target cells and subsequent viral replication processes. A high DENV concentration in the blood has been shown to be significantly correlated with disease severity [7]. These reports provided the only explanation of CD209 genetic diversity among DENV infection patiens.

The role of the specific binding site of DENV with the CD209 CRD region in the wide spectrum of clinical manifestions among patients needs to be further explored since this area is not polymorphic. There was no report about the frequency of the SNPs analyzed in this study in other ethnic groups and races, but it was reported in the ExAC database that the allele frequency of these SNPs was less than 0.01% [16]. However, dbSNP showed that the allele frequency of rs11465393 was as low as 2.5% for T allele in the European population and 1.1% in the Asian population. There were no data for the other four SNPs. Indeed, the small sample size in this study should be taken into account when drawing general conclusions about Indonesian DENV-infected patients.

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