

# Dengue Virus Nonstructural-1 Protein And Its Phylogenetic Correlation To Human Fibrinogen and Thrombocytes: A Study To Explain Hemorrhagic Complications

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## Citation

V Wiwanitkit. *Dengue Virus Nonstructural-1 Protein And Its Phylogenetic Correlation To Human Fibrinogen and Thrombocytes: A Study To Explain Hemorrhagic Complications*. The Internet Journal of Genomics and Proteomics. 2003 Volume 1 Number 2.

## Abstract

Dengue infection is a major public health problem, yearly affecting thousands of children in the tropical countries. Luckily, the classical form of this infection resembles to viral flu: fever, headache, chill and rash. Mostly, affected patients have a complete recovery without any complication. However, there is a severe form of dengue infection called dengue hemorrhagic fever. In this case, the host immunological response host is the important factor in the course of the disease. However, the mechanisms underlying severe bleeding in dengue DHF are not completely understood. Recently, it was noted that the dengue virus nonstructural-1 protein (NS1) generated antibodies to common epitopes on fibrinogen and integrin/adhesin proteins on the platelet. Therefore, the role of NS1 as an important protein causing the unwanted immunological reaction bringing DHF is hypothesized. In this article, the author performs a phylogenetic analysis to answer the question how dengue virus NS1 protein is related to fibrinogen and platelet integrin/adhesin relating proteins. Answering this question, the author performed a multiple sequence alignment phylogenetic tree to present the family tree of the dengue virus NS1 proteins, fibrinogen and platelet integrin/adhesin relating proteins recorded in the genomic databases. These derived sequences from database were processed by ClustalW and subsequently used for preparation of distance matrix by Phylip protdist. The final generated phylogenetic tree of the studied proteins was presented. According to this study, it can be shown that D-2VNS1 presents more homology to those four related human proteins than D-3VNS1, possibly explaining the more common of dengue type 2 than dengue type 3 virus in DHF. In addition, the dengue virus NS1 protein presents the closed phylogenetic correlation to CD 61 than fibrinogen and the other two platelet integrin/adhesin relating proteins (CD41 and CD49B). Therefore, platelet CD61 should be further studied in the pathogenesis of DHF.

## INTRODUCTION

Dengue infection is a major public health problem, yearly affecting thousands of children in the Southeast Asia Region [1]. Luckily, the classical form of this infection resembles to viral flu: fever, headache, chill and rash. Mostly, affected patients have a complete recovery without any complications [2,3,4]. However, there is a severe form of dengue infection called dengue hemorrhagic fever. In this case, the host immunological response host is the important factor in the course of disease [5]. These responses are immune complex formation, complement activation, increase histamine release and a massive release of many cytokines into the circulation, leading to shock, vasculopathy, thrombopathy and disseminated intravascular coagulation (DIC) [5].

However, the mechanisms underlying severe bleeding in dengue DHF are not completely understood. Recently,

Falconar proposed that the dengue virus nonstructural-1 protein (NS1) generated antibodies to common epitopes on human blood clotting and integrin/adhesin proteins on the platelet [6]. Falconar found that anti-NS1 polyclonal antisera reacted with the NS1 proteins of the dengue virus, but only weakly reacted with the NS1 proteins of the other flaviviruses [6]. In addition, several anti-NS1 monoclonal antibodies produced haemorrhage in mice, cross-reacted with human fibrinogen, thrombocytes and endothelial cells, with known epitopes or active sites on human clotting factors and integrin/adhesin proteins present on these cells [6]. Therefore, the role of NS1 as an important protein causing the unwanted immunological reaction bringing DHF is hypothesized. Here, the author performs a phylogenetic analysis to answer the question how dengue virus NS1 protein the related to fibrinogen and platelet integrin/adhesin relating proteins. Answering this question, the author

performed a multiple sequence alignment phylogenetic tree to present the family tree of those proteins.

**MATERIALS AND METHODS**

The database Pubmed (<http://www.pubmed.com>) and ExPASy [7] was used for data mining of the protein sequences of dengue virus NS1 proteins, fibrinogen and platelet integrin/adhesin relating proteins. The derived sequences of all proteins were aligned using ClustalW [8]. The ClustalW alignment file of the selected sequences was used for the basic parameter for further creating of the phylogenetic tree.

Distance matrix for the ClustalW alignment file of the selected sequences were made using the Phylip protdist program [9] using Felsenstein's "categories" distance; all other parameters were kept at the default. The Phylip neighbor program was used to generate a neighbor-joining tree from each other of the distance matrixes. Trees were drawn using Phylo dendron by D.G. Gilbert version 0.8d (<http://www.es.embnct.org/>)

**RESULTS**

From searching of the databases, human Integrin alpha-2 precursor (CD49B), human Integrin alpha-IIb precursor (CD41), human Integrin beta-3 precursor (CD61), fibrinogen, dengue-2 virus NS1 nonstructural protein (D-2VNS1) and Dengue-3 virus NS1 nonstructural protein (D-3VNS1) were derived as shown in Table 1(in March 2004). These sequences were processed by ClustalW and subsequently used for preparation of distance matrix by Phylip protdist. The distance matrix showing the relative number of difference in sequences of these proteins was presented as Table 2. The final generated phylogenetic tree of these proteins was shown in Figure 1.

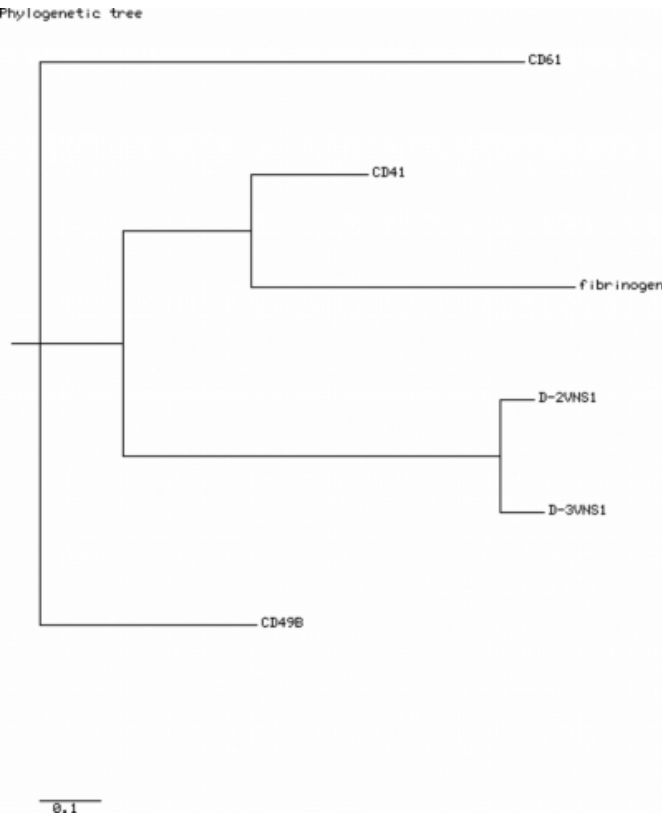
**Figure 1**

Table 1: Human platelet glycoprotein sequences according to the databases.

Gene name	Description
D-2VNS1	dengue-2 virus NS1 nonstructural protein
D-3VNS1	dengue-3 virus NS1 nonstructural protein
CD49B	human Integrin alpha-2 precursor
CD41	human Integrin alpha-IIb precursor
CD61	human Integrin beta-3 precursor
fibrinogen	fibrinogen

**Figure 2**

Figure 1: Phylogenetic tree of studied proteins



**DISCUSSION**

In 1995, Wang et al reported the first evidence that dengue type 2 virus binds to human platelets only in the presence of virus-specific antibody, supporting a role for immune-mediated clearance of platelets in the pathogenesis of thrombocytopenia in DHF [10]. It is mentioned that antibody responses generated by mice to the dengue NS1 protein were influenced by MHC class II (I-A) haplotype but each antiserum cross-reacted with human fibrinogen, thrombocytes and endothelial cells [6]. Therefore, the homology of NS1 and other mentioned proteins can be a potential role of both antigenic and biochemical mimicry in dengue haemorrhagic fever pathogenesis, consistent with clinical data [6].

Here, the author used the phylogenetic analysis technique to clarify the correlation between dengue virus NS1 protein and fibrinogen and platelet integrin/adhesin relating proteins. A closed relation among these proteins can be seen (Table 2). It can be shown that D-2VNS1 presents more homology to those four related human proteins than D-3VNS1. Indeed, the dengue type 2 virus is reported to be more common than dengue type 3 virus in DHF [11]. The resulted homology

pattern in this study might be an explanation for this observation.

### Figure 3

Table 2: The distance matrix showing the relative number of difference in sequences of proteins.

	CD49B	CD41	CD61	fibrinogen	D-2VNS1	D-3VNS1
CD49B	0.0000	2.0579	3.2326	3.6365	3.5870	3.3017
CD41	2.0579	0.0000	4.0454	2.0350	3.1673	3.2603
CD61	3.2326	4.0454	0.0000	4.6893	4.2110	4.5673
Fibrinogen	3.6365	2.0350	4.6893	0.0000	3.8452	3.8465
D-2VNS1	3.5870	3.1673	4.2110	3.8452	0.0000	0.3598
D-3VNS1	3.3017	3.2603	4.5673	3.8465	0.3598	0.0000

In addition, the dengue virus NS1 protein presents the closed phylogenetic correlation to CD 61 than fibrinogen and the other two platelet integrin/adhesin relating proteins (CD41 and CD49B) (Table 2, Figure 1). Indeed, Chang et al reported that dengue NS1 immobilized on coverslips resulted in more cell adhesion than did the control proteins and indicated that integrin – relating peptides structural mimicry existed within the NS1 antigen [12]. Therefore, platelet CD61 should be further studied in the pathogenesis of DHF.

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