

Phylogenetic and Conservational Analyses of Dengue Non-Structural Protein 1

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Abstract— *The variability in the host immune responses against infections by different strains of Dengue virus (DENV), due to their sequence heterogeneity, is a huge obstacle in disease management and development of an effective vaccine. In depth understanding of genetic diversity among different serotypes and strains is therefore an important factor to manage the disease. This study is an attempt to analyze the phylogenetic diversity of dengue Non-Structural protein 1 (NS1), which has a significant functional role in dengue pathogenesis. Fifty sequences of NS1, each from the four dengue serotypes, Japanese Encephalitic Virus (JEV) and West Nile Virus (WNV) were obtained from NCBI Genebank, were phylogenetically aligned on MEGA6. The level of conservation of the sequences was analyzed by IEDB analysis tools and WebLogo. The sequences from each strain were selected representing a broad time span and multiple regions of the world. In the phylogenetic tree, strains of each DENV serotype were grouped into four main geographical locations, Africa, South America, South East Asia and China. DENV 1 and 3 were phylogenetically closer among the four serotypes. They further showed more than 80% conservation between each other. DENV 4 showed a higher deviation from other three serotypes. Strains from DENV3 showed high level of sequence conservation, while it was highly variable within DENV 4. Dengue group showed a conservation level of 50% in NS1 protein with JEV and WNV. However, the carboxy terminal region of the protein (271-352aa) appeared more conserved across different flavivirus groups, showing a conservation level of more than 70%. The understanding of genetic variability or similarity among and within the serotypes of dengue virus would provide insight into better management of the disease.*

Keywords— *Dengue, NS1 Protein, Conservation*

I. INTRODUCTION

Dengue is a mosquito-borne viral disease that has rapidly spread in all regions of the world. The disease is widespread throughout the tropical and sub-tropical countries, with local variations in risk influenced by rainfall, temperature and unplanned rapid urbanization.

The incidence of dengue has grown dramatically around the world in recent decades. One recent estimate indicates 390 million dengue infections per year. Not only is the number of cases increasing as the disease spreads to new areas, but explosive outbreaks are occurring. The threat of a possible outbreak of dengue fever now exists in Europe. An estimated 500 000 people with severe dengue require hospitalization each year, a large proportion of whom are children. About 2.5% of those affected die (Bhatt, *et al* 2013; Guzman, *et al* 2010).

Dengue virus (DENV) is a RNA virus of the family Flaviviridae and exists as four closely related antigenically distinct serotypes denoted DENV-1 to DENV-4. Genetic variation within and among each of these four serotypes has been observed to cause implications for pathogenesis of DENV infection, variable disease outcomes, virus evolution, and highly variable host immunity (Clyde, *et al* 2006).

The dengue virus has a genome of about 11000 bases that encodes a single large polyprotein that is subsequently cleaved into several structural and non-structural mature peptides. The polyprotein is divided into three structural proteins, capsid, pre membrane, envelope; seven nonstructural proteins, NS1, NS2a, NS2b, NS3, NS4a, NS4b, NS5; and short non-coding regions on both the 5' and 3' ends (Guzman, *et al* 2010)

Although it is a nonstructural protein, NS1 has also been observed to elicit strong humoral immune responses in mammalian cells (Muller, *et al* 2013). The reason being that it is expressed in three forms: an endoplasmic reticulum (ER)-resident form, a membrane-anchored form, and a secreted form (Muller, *et al* 2013; Winkler, *et al* 1989). The secreted form of NS1 (sNS1) is responsible for this observed anti-NS1 antibody production. NS1 protein has 354 amino acids and it is a glycoprotein. In DENV-infected mammalian cells, NS1 is synthesized as a soluble monomer and is dimerized after posttranslational modification in the ER where it plays an essential role in viral replication (Guzman, *et al* 2010). Antibodies against

this secreted NS1 protein has also been suggested as a contributing factor to vascular leakage associated with severe dengue disease.

With the absence of licensed vaccines or specific antiviral therapies for dengue, patient management relies on good supportive care. Prompt and early diagnosis of dengue viral infection remains crucial. Laboratory confirmation is important due to difficulties in making accurate diagnosis due to the broad spectrum of clinical presentations. Among the available dengue diagnostic tools, the detection of virus encoded NS1 antigen has become the basis for commercial diagnostic kits and laboratories are increasingly using NS1 detection as the preferred diagnostic test

Therefore the proper understanding of the genetic variation underlying dengue virus proteins are crucial in better management in the disease, and even in the development of an effective dengue vaccine.

II. METHODOLOGY

Retrieving NS1 protein sequences

Polyprotein sequence of the four distinct Dengue serotypes, JEV and WNV were obtained from NCBI gene bank. NS1 protein sequences of the Dengue virus (354 amino acids) was identified from the whole protein sequences and were aligned using MEGA 6 software. Fifty sequences from each serotype were selected to represent different geographical areas of the world and a wider time span.

Conservation analysis and phylogenetic analysis

Conservation of the whole NS1 sequence was analyzed by using weblogo analysis tool (weblogo.berkeley.edu) and conservation analysis tool available at IEDB Resource. The phylogenetic tree of the whole NS1 sequence was generated using the MEGA 6 program.

III. RESULTS AND DISCUSSION

The levels of conservation between the NS1 whole protein sequences of the four dengue serotypes and WNV or JEV were compared pair wise (Table 1). The level of conservation across the three flavivirus groups was approximately 50% (Table 1). The flavivirus groups, JEV and WNV, showed a conservation level of more than 75%, between each other. Interestingly, the carboxy terminal region of NS1 protein (271-352aa) (Chen, *et al* 2009) appeared more sequence conserved across the three flavivirus groups, which was observed to have a level of more than 70%. This region has been suggested to produce cross reactive antibodies with vascular epithelium, has been proposed to be contributing to vascular leakage associated with severe dengue disease.

Dengue serotypes 1 and 3 seemed to have a closer genetic relationship with each other, as compared to other two serotypes, showing 80% conservation between each other. Dengue type 3 was more conserved (95%) within the serotype than the other three serotypes (less than 90%). That was the lowest in serotype 4 (within serotype conservation level 80%).

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Table 1. Conservation analysis between dengue serotype pairs and other flavi viruses

Dengue type	D1	D2	D3	D4	JEV	WNV
D1	-	73%	80%	68%	51%	49%
D2	73%	-	74%	71.5%	53%	55%
D3	80%	74%	-	73%	52%	52%
D4	68%	71.5%	73	-	51%	53%
JEV	51%	53%	52%	51%	-	77%
WNV	49%	55%	52%	53%	77%	-

Legend: The conservations ranged from a minimum percentage up to a maximum of 100%. The indicated percentages are the minimum conservations. D1- Dengue type 1, D2- Dengue type 2, D3- Dengue type 3, D4- Dengue type 4, JEV- Japanese ensapalities virus, WNV- West Nile virus

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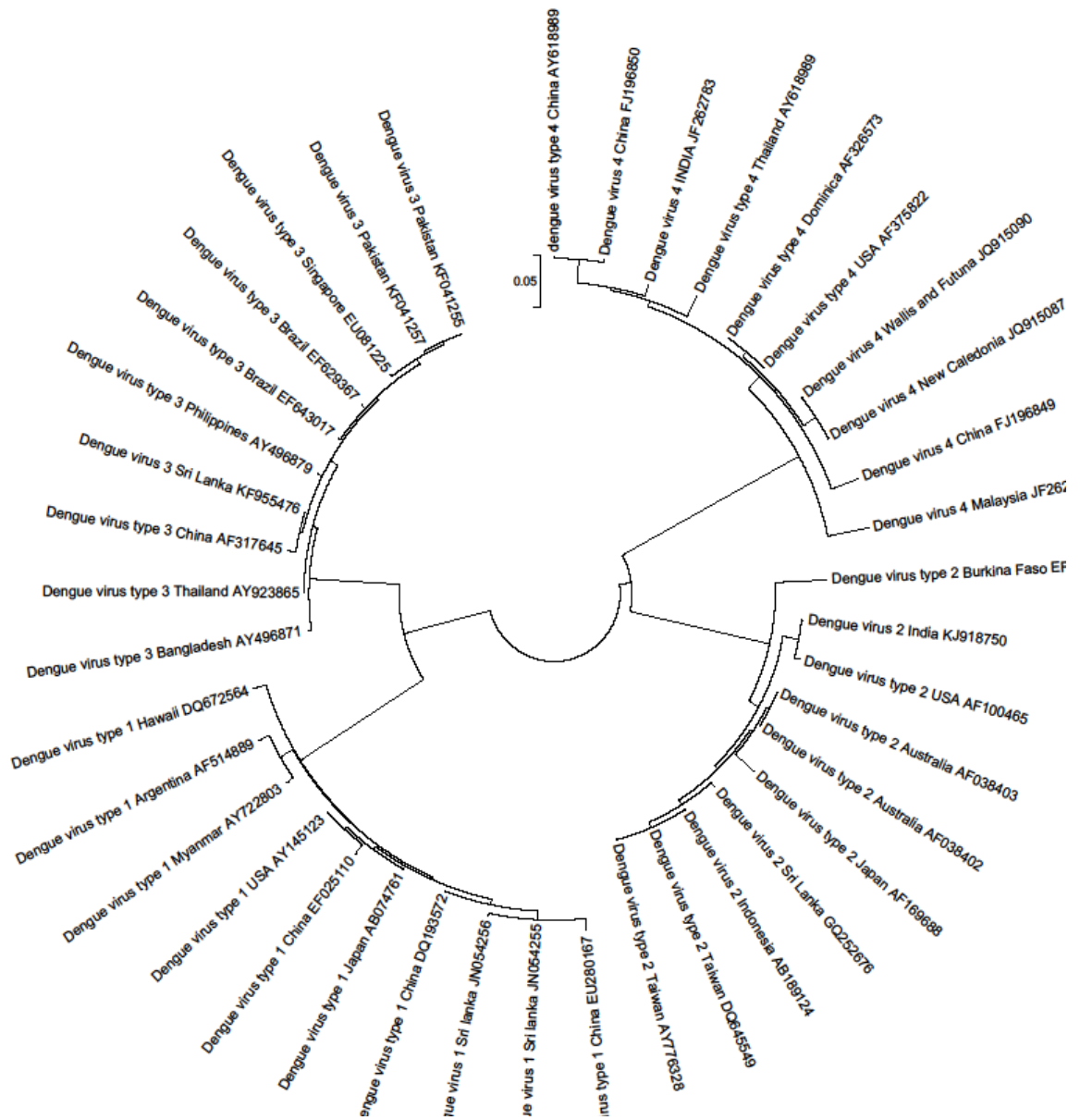


Figure 1: Phylogenetic tree of the NS1 protein of Dengue, JEV and WNV

The phylogenetic tree generated with whole NS1 sequences of dengue virus are shown in figure 1. Accordingly, the serotypes 1 and 3 seem phylogenetically more closely related (Figure 1). The serotype 2 has branched off before serotypes 1 and 3 have separated. Serotype 4 was the most distant from the other serotypes, and appears as an isolated group in the phylogenetic tree. In line with the results from conservation analysis, the genetic diversity of the strains within the serotype 4 appeared higher than what was observed within other three serotypes.

Sequence heterogeneity among the strains from a given serotype shows additional levels of organization into subtypes within a given serotype. This was also evident in the phylogenetic tree. In agreement with the results from conservation analysis, the strains of dengue serotype 3 appeared to have organized into lesser number of subgroups within the serotype showing a higher level of conservation within the particular serotype. However, there appeared to have a higher number of subgroups within serotype 4, reflecting high variability in the strains from serotype 4. Further, according to this phylogenetic analysis all dengue strain appears to have segregated into four main geographical regions of the world. They are namely, South America, South Asia, Africa and East Asia. Strains isolated from Sri Lanka are localized with other South Asian strains

IV. CONCLUSION

Dengue virus has more diversity except diversity between main four serotypes. Because of that dengue virus has subtypes within the serotype. Dengue type 4 has more subtypes than other three types and dengue type 3 has less number of subtypes. Dengue type 1 and 3 have close relation according to the both conservation and phylogenetic analysis. All four dengue serotypes could be grouped into four groups according to the geographical distribution. They were, South America, Africa, South Asia and East Asia. Sri Lankan dengue strains grouped with South Asian group.

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