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MINI-REVIEW

Genomic Basis of Dengue Virus: Drawing the Puzzle of Dengue Viral Infection

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ABSTRACT

Dengue fever is an epidemic killing millions of every year around the world especially in regions with moderate temperature primarily due to propagation of the vector, *Aedes aegypti*. Unknown genomic basis is the biggest challenge to compete with this challenge. A little information is available about the genotypic characterization of the virus infecting the population. Based on the genotype, dengue viruses are classified into four types named as DENV-1, DENV-2, DENV-3, DENV-4. All these serotypes are composed of several proteins know as NS proteins which are further categories into NS1, NS2A, NS2B, NS3, NS4A,NS4B, NS5 based on their functions. In this manuscript, detailed of serotypes classification, their components proteins, their structure and functions are discussed briefly which will be helpful to understand the basic of dengue viral genome.
Keywords: Dengue viral infection, Viral borne diseases, DENV serotypes, NS proteins

Dengue viral infection is one of the most common mosquito borne infectious diseases in the world caused by dengue virus (DENV), a group of four flaviviruses which are known as *serotypes* and are named as dengue virus type 1 (DENV-1), dengue virus type 2 (DENV-2), dengue virus type 3 (DENV-3) and dengue virus type 4 (DENV-4) (1). *Flavivirus* is the genera of *Flaviviridae* family having *Pestivirus* and *Hepacivirus, other genera*.
The dengue virus, like those of other flaviviruses, is spherical and 40-50 nm in diameter. It is composed of a 30 nm nucleocapsid enclosed in a lipid envelope which contains the viral capsid and RNA genome. The lipid-containing envelope consists of a lipid bilayer, an envelope protein between 51,000 and 59,000 Da responsible for the attachment, fusion, and penetration of virus in association with small non-glycosylated internal matrix protein of approximately 8,500 Da. The envelope protein is glycosylated in most flaviviruses and is exposed on the virion surface. Electron microscopy studies have shown that mature dengue virions are characterised by a relatively smooth surface, with 180 copies of the envelope protein forming the icosahedral scaffold (2) as shown in figure 1.

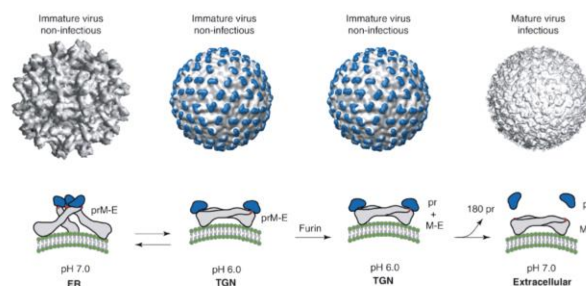

Figure 1. Structure of the dengue virion and conformations of the E protein (2). (ER: endoplasmic reticulum; TGN: trans-Golgi network; prM: precursor of membrane)

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The genomic organization of the dengue virus, and by extension all flaviviruses, is relatively simple as compared to other arboviral families such asthe *Togaviridae* (formerly known as group arboviruses), *Bunyaviridae* or Rhabdoviridae. The DENV genome consists of a single-stranded, positive senseRNA molecule roughly 10.7 kb in size. It contains a single translatedopen reading frame (ORF) that *encodes* a precursor polypeptide of around 3390 amino acids which is processed catalytically into ten viral proteins. There is no evidence of substitute or overlapping reading frames thatare translated and there is also no hyper-variable region in the DENV genomelike those reported in the HCV genome.

The DENV ORF is flanked at its 5' terminus by an untranslated region (UTR) of about 100 nucleotides and a longer UTR of about 500 nucleotides atits 3' terminus. The 5' terminus of the genome has a type I cap (m7GpppAmp)and there is no polyadenylation of the 3' terminus. The translated polyprotein is cleaved co- and post-translationally byviral and host proteases into ten viral proteins: three structural proteins (C,capsid; prM/M, precursor of membrane; E, envelope) encoded at the 5' end ofthe ORF, and seven non-structural proteins (NS1, NS2A, NS2B, NS3, NS4A,NS4B and NS5) encoded at the 3' end.The three structural proteins constitute the DENV virion: the capsid protein surrounds the viral RNA genome to form the nucleocapsid, whereas the prM and E proteins are embedded in the lipid bilayer that forms the viral envelope. Cleavage of the prM into the membrane (M) protein by furin during viral release has been shown to be a prerequisite for the production of mature infectious virions. Of the three structural proteins, the E protein is the most studied as it is the major constituent of the virus envelope. It is glycosylated at two sites (Asn-67 and Asn-153) and is responsible for virus attachment toreceptors of susceptible host cells and for fusion with cell membranes. The Eglycoprotein also contains the main epitopes recognized by neutralizing antibodies. Such epitopes are also foundto a lesser extent on the M glycoprotein (3).

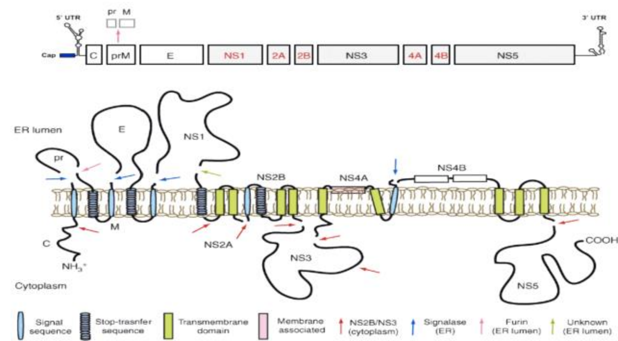

Figure 2. Gene organization in the dengue virus RNA genome, and the bottom cleavage sites of the transcribed polyprotein. Cellular and viral proteases, which are denoted by arrows, process the immature polyprotein into ten separate proteins (3)

NS1 (non-structural protein 1) is a highly conserved glycoprotein that is essential for the viability of DV and is produced both in membrane-associated and secretory forms by the virus.

Table 2. Known and possible functions of dengue non-structural Proteins (3).

NS proteins	*Description of known functions*
NS1	Plays a role in viral RNA replication complex; acts as Soluble complement-fixing antigen.
NS2A	Forms part of the RNA replication complex.
NS2B	Co-factor for NS3 protease.
NS3	Serine protease, RNA helicase and RTPase/NTPase.
NS4A	Possibly induces membrane alterations important for virus replication.
NS4B	Possibly blocks IFN α/β-induced signal transduction.
NS5	Methyltransferase (MTase) and RNA-dependent RNA polymerase (RdRp).

Enzyme-linked immunosorbent assays (ELISA) directed against NS1 antigen (NS1 Ag) have demonstrated its presence at high concentrations in the sera of DV infected patients during the early

clinical phase of the disease(4). The detection of secretory NS1 protein represents a new approach to the diagnosis of acute DV infection.

The role of NS1 Ag for early detection of DV infection is currently being evaluated by many investigators, without the requirement of paired sera (5, 6). NS1 Ag circulates uniformly in all serotypes of dengue virus and it circulates at high level during the first few days of illness(7). NS1 Ag levels varies from 0.04 - 2 μg/ml in acute-phase serum samples, to only 0 .04μg/ml or even less in convalescent phase serum (8). This is the reason for its higher detection rate in acute phase sera. The morbidity and the mortality of DHF can be reduced by early diagnosis, hospitalization and symptomatic care. Thus NS1 assay expedited the laboratory confirmation within the first four days of illness. Some studies claim that in addition to an early diagnosis, NS1 antigen may be an indicator of disease severity. (Bessof *et al*, 2008).

A very high concentration of NS1 antigen was observed within 72 hours of illness identified patients at risk of developing DHF (9) though a quantitative estimation of NS1 Ag was not carried out in this study to confirm this observation. 66.66% were NS1 Ag positive. All the positive patients were classified as DF and they were all detected by NS1 Ag assay within the first four days of fever. Their platelet count remained normal throughout the illness. The assay could diagnose 66.6% cases of DF in the first four days of illness. The sensitivity of this assay was good, especially in samples collected from cases classified as DF. Samples from cases that were negative by NS1 Ag assay presented as undifferentiated fever and were subsequently screened for antibodies after five days of fever. All tested negative confirming the high specificity of this assay. The specificity of NS1Ag assay was also assessed from the control group which was 100%.

The conclusion is, NS1 Ag assay is an effective tool for diagnosis of DV infection, especially within the first four days of illness. Early detection of DHF by NS1 assay can help in early confirmation and management of this vulnerable group. NS1 Ag assay, if used in combination with MAC-ELISA on a single serum sample of a suspected case, has the ability to improve the diagnostic algorithm contributing significantly to the clinical treatment and control of dengue viral infections.Based on available molecular data it is well known that there is great genetic diversity between the dengue viruses. The factors that contributed to this are many fold, so are the epidemiological implications arising from this assortment.

Each serotype of the dengue virus can be further classified into several genetic groups called *genotypes* (the term *subtype* is used interchangeably) based on sequence diversity. (10) initially defined a dengue genotype as a group of dengue viruses having no more than 6% sequence divergence within a 240-nucleotide region of the DENV-1 and DENV-2 E/NS1 junction. Since then, both the length and region of virus genome selected for sequencing varied greatly depending on research groups, ranging from the complete sequence of single genes to the complete genome of the DENV. Assignment of genotypes now depends on phylogenetic analysis to a certain extent than random cut-off values in sequence diversity. (11) have published brilliant and detailed descriptions of the genotype classification for all four dengue serotypes. The following paragraphs describe only the essential points of the subject matter.DENV-1 can be divided into five genotypes based on the complete E gene sequence as described by (12). Earlier work by (10) also classified DENV-1 into five groups based on the 240-nucleotide E/NS1 junction sequences, but with some minor differences from the newer scheme.

The current genotype classification for DENV-3 follows the nomenclature proposed by (13) which recognized four DENV-3 genotypes based on prM/E sequences as shown in table 6. These four genotypes are similar to the four groups described by (14) using a 195-nucleotide region at the 5' terminus of the E gene. Introduced to the Americas via Nicaragua in 1994, genotype III DENV-3 is now widely found in Central and Southern America (15-17)and is considered as the most virulent of the four DENV-3 genotypes. It is worthy of note that genotype IV has never been associated with any DHF epidemics (13).Although their existence is anticipated through the presence of DENV-3 antibodies in non-human canopy-dwelling primates, no sylvatic lineage of DENV-3 has been found thus far.

A study initially separated DENV-4 into two genotypes, I and II, based on the complete E gene sequence. A further two genotypes were subsequently found only in non-human primates in Malaysia and another, genotype III, found only in Bangkok, Thailand (18). Genotype II DENV-4 is the most widespread of the four following an introduction to the Western hemisphere in 1981, possibly via the Pacific islands (19, 20). Although DENV-4 is the least frequently sampled serotype, it is often associated with haemorrhagic fever during secondary infection (21). Except for the sylvatic genotypes, genotype classification can often unveil the geographical origin of the dengue virus strains. This has enabled tracking the route of virus transmissions across distant time and place, and has served as the basis of molecular epidemiological studies that can determine whether dengue epidemics are caused by introduction of new viruses or the result of re-emergence of endemic strains.

Introduction of new viruses inevitably leads to the question whether particular genotypes of DENV are associated with higher virulence or severe disease. To date, severe disease has often been associated with several DENV genotypes originating in Southeast Asia (22, 23). The lack of a suitable animal model for the dengue disease, however, means such hypotheses cannot be easily verified (11). On the other hand, association of re-emergence of endemic strains with outbreaks leads to a different question that can only be answered by a combination of classic epidemiology and comparative genomics: whether the viruses re-emerged due to environmental, population immunity and/or vectorial factors, or whether outbreaks were triggered by adaptive evolution of the virus that endowed it with an increase in fitness and virulence?

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