Investigation of polymorphisms in the genome of dengue virus

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Abstract

In the present study, we investigated the nucleotide polymorphisms among the four genotypes of the Dengue virus - DENV-1, DENV-2, DENV-3, and DENV-4 - by qualitative and quantitative characterization. We studied the Nucleotide Substitution Density (NSD) along each genome identifying the regions with higher degree of mutation and/or conservation. Then, we calculated the Average Nucleotide Substitution Density (ANSD) for each serotype. We observed that the ANSD of DENV-2 is larger than for DENV-1 by 44.21%, DENV-3 by 85%, and DENV-4 by 163.31%. In contrast to DENV-2 and DENV-4, DENV-1 and DENV-3 showed a similar mutational behavior. The NS5 domain from DENV-2 that corresponds to the RNA-dependent RNA polymerase also has a higher mutation rate compared to that of the other DENVs. This suggests that the polymorphism and the virulence can be correlated in DENV-2, which could contribute to the understanding of the disease evolution.

Keywords

Dengue, NS5, polymerase, virulence, polymorphism
Introduction

The Dengue virus (DENV) is the most important flavivirus that causes human disease in Brazil (Figueiredo, 1998). Because Dengue is potentially fatal and has a fast geographical dissemination, it attracted the attention of the Brazilian public authorities in the 90s. The simultaneous presence of two or more Dengue serotypes in the same area - hyperendemicity - increases the risk for complications, such as hemorrhagic fever (FHD) and shock syndrome (SCD), caused by an exaggerated immunological response of the host. Therefore, it is essential to understand how viral polymorphisms can influence the pathogenicity (GUBLER, 1997; 1998).

Dengue is the arbovirus with the largest diffusion in the world. It is found in tropical and subtropical areas where approximately 3 million people are upon risk of infection. Dengue has been identified in more than 100 countries and 2.5 billion people are living in endemic areas (GUZMAN; KOURI, 2002). 50-100 million cases with hundreds of thousands severe cases of the disease (FHD/SCD) and thousands of deaths (about 25,000/ year) are reported each year depending on the viral epidemic activity. Dengue has 4 distinct serological types: DENV-1, DENV-2, DENV-3 and DENV-4. They differ in antigenicity and do not induce cross protection, but possible to establish a clear correlation between one sero-type or a particular genotype of DENV and the severity of an epidemic, some DENV-2 and DENV-3 genotypes were associated with DHF. In general, Asian genotypes were correlated with DHF while the Asian genotypes were correlated with FHD (CLYDE et al., 2006).

The origins of Dengue viruses circulating in Brazil were determined based on the phylogeny of nucleotide sequences. DENV-1 was found to derive from the Caribbean islands while isolates from DENV-2 originated more specifically from Jamaica. Both viruses were probably introduced through the Caribbean (MIAGOSTOVICH et al., 1998; WALNUT et al., 1991). DENV-3 was isolated for the first time in Brazil in 1999 from a patient who was returning from travel in Nicaragua (FIGUEIREDO, 2000).

The majority of FHD/SCD cases were associated with secondary and heterotypical infections, especially during the DENV-2 epidemic with DENV-1 background (FIGUEIREDO, 2000). The successive epidemics of Dengue in Brazil, caused by DENV-1 and DENV-2, culminated with FHD/SCD occurrences. With the continuous circulation of the two serotypes 1 and 2 together, and with the additional high risk of the introduction of other types, Dengue became an important health problem in Brazil (FIGUEIREDO, 2000). Although it has not been possible to establish a clear correlation between one serotype or a particular genotype of DENV and the severity of an epidemic, some DENV-2 and DENV-3 genotypes were associated with DHF. In general, Asian genotypes seem to be more virulent than those initially found in the Americas and in the South Pacific (MESSER et al., 2003; RICO-HESSE et al., 1997; WATTS et al., 1999). In the case of DENV-2, phylogenetic analyses showed that only the American native genotypes were associated with FHD while the Asian genotypes were correlated with FHD (CLYDE et al., 2006).

Materials and Methods

A total of 3,278 dengue viral sequences were extracted from the public database EMBL (European Molecular Biology Laboratory), comprising 953 of DENV-1, 1,077 of DENV-2, 1,167 of DENV-3 and 531 of DENV-4. The SRS LION system (Sequence Retrieval System, Release 7.1.3.2) was used to retrieve the sequences at EBI (European Bioinformatics Institute, http://srs.ebi.ac.uk/).

The local processing of files and sequences was performed with Perl scripts. Sequences of each serotype were treated separately. We first eliminated the sequences recognized as part of larger fragments, retaining only the largest non-redundant sequences. Then, the sequences of each serotype were globally aligned one by one with the ClustalW program (http://www.ebi.ac.uk/Tools/clustalw/index.html), using a complete genome sequence of the corresponding serotype as a reference. The reference sequences for the 4 DENVs were selected in the NCBI database (National Center for Biotechnology Information - http://www.ncbi.nlm.nih.gov/). The consistency of each alignment was verified by visual inspection using the program Seaview (http://pbil.univ-lyon1.fr/software/seaview.html).

We calculated the degree of polymorphism of each serotype by counting in each column of the multiple global alignment (~10,700) the number of nucleotides that differ from the corresponding nucleotide in the reference. In other words, if mi is the number of aligned sequences in the i-th column and nri is the amount of nucleotides that match the nucleotide of the reference sequence in that column, then the degree of polymorphism in the i-th position is given by: pi = (mi - nri)/mi. The graph of pi vs i presents a dense succession of peaks that makes the
interpretation difficult (data not shown). Given this, we introduced the function Nucleotide Substitution Density \( N_{SD} = \frac{1}{L} \sum_{j=1}^{L-1} \frac{N}{j} \). NSD is defined for a uniform genome partition in segments of length L. In this study, we selected a length interval of 100 bases that allows smooth curves of NSD along the genome (Figure 1). The graph of NSD for the four serotypes was constructed with the program Scilab 4.1.1 (http://www.scilab.org) using the function smooth that carried out a cubical smoothing by spline. The number of aligned sequences varied with the genome position and the serotype. The number of sequences in the region of the envelope was approximately 5 times larger, in average, than in the remaining intervals. The number of sequences outside the envelope region was never below 100. In order to ensure comparison consistency for each serotype, we calculated the Average Nucleotide Substitution Density ANSD as \( ANSD = \frac{1}{N} \sum NSD \).

**Results and Discussion**

After eliminating the redundancy among the 3,278 Dengue sequences retrieved from EMBL, the sequence samples were 757 for DENV-1, 848 for DENV-2, 866 for DENV-3 and 398 for DENV-4. Hence, the size of these samples is large enough to be representative and ensure statistical significance in nucleotide polymorphism analyses.

The curves of the degree of nucleotide polymorphism along the genome for the 4 DENV serotypes are shown in Figure 1. We observed that serotypes 1 and 3 present similar polymorphism profiles, but that they differ substantially from the profiles of serotypes 2 and 4. However, the latter two serotypes differ much more from one another. The Average Density of Nucleotide Substitution of DENV-2 was significantly larger than that of the other serotypes, i.e. 44.21% larger than DENV-1, 85% larger than DENV-3 and 163.31% larger than DENV-4. The region encoding for the envelope protein E presented the highest amount of aligned sequences (5 times more) (data not shown). In this study, we further investigated the NS5 region that encodes the RNA polymerase (ACKERMANN; PADMANABHAN, 2001; NOMAGUCHI et al., 2003; TAN et al., 1996; Egloff et al., 2002). In relation to nucleotide substitutions in this interval, we observed two different patterns: the first one is shared by DENV-1 and DENV-3 and the second is common to DENV-2 and DENV-4, although DENV-2 has a significantly larger level of nucleotide substitutions than the other serotypes. One may speculate that polymerase alteration could promote increase in the mutation rate and explain the largest degree of polymorphism observed in DENV-2. Such hypothesis is currently being investigated by molecular modeling at the protein level. Moreover, since the largest variability within a serotype is associated with the largest degree of mutation, it is possible to think that it could be related to increased virulence and adaptability. The virulence, together with the host susceptibility and environment factors, account for the viral pathogenicity. Amongst these, virulence appears to be a factor obviously dependent upon the genome sequence. Given the existence of four serotypes of Dengue with the same genomic structure, it is possible to presume that the difference in pathogenicity has a functional cause that finds its origin in the sequence. Nucleotide polymorphism analyses help us to identify genomic regions that could be related to Dengue virus virulence.

![Figure 1](image-url) - Density of Nucleotide Substitution along the genome sequence for each serotype of Dengue. The genome was divided in intervals of 100 bases to compute the nucleotide substitution density. The curves were smoothed with spline (program Scilab 4.1.1).
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Bibliographic references
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