Infection, Genetics and Evolution 43 (2016) 130–134

Contents lists available at ScienceDirect

Infection, Genetics and Evolution

journal homepage: www.elsevier.com/locate/meegid

Research paper

Phylodynamics of DENV-1 reveals the spatiotemporal co-circulation of two distinct lineages in 2013 and multiple introductions of dengue virus in Goiás, Brazil

Marielton dos Passos Cunha, Vanessa Neiva Guimarães, Menira Souza, Divina das Dôres de Paula Cardoso, Tâmera Nunes Vieira de Almeida, Thaís Santana de Oliveira, Fabíola Souza Fiaccadori *

Federal University of Goiás, Goiânia, Goiás, Brazil

ARTICLE INFO

Article history:
Received 8 December 2015
Received in revised form 12 May 2016
Accepted 16 May 2016
Available online 17 May 2016

Keywords:
Dengue virus type 1
Lineages
Genotype V
Phylodynamics
Phylogeography
Phylogeny

ABSTRACT

Dengue virus type 1 (DENV-1) was the first serotype introduced in Brazil, during in the 1980s. Since then, this virus has spread in the Brazilian territory, causing several outbreaks. In 2013 the highest number of dengue cases was notified, when compared to the previous years in Brazil, and the state of Goiás reported over 160 thousand cases. In this study, we aimed to present the Phylodynamics of DENV-1 isolates from the state of Goiás, Brazil, during 2013 outbreak, based on the envelope gene (E) sequences. Phylogenetic analysis revealed that Brazilian DENV-1 isolates are grouped together with viruses from genotype V in two distinct lineages (lineage I and lineage II) reflecting co-circulation. Phylogeographic analyses showed that these lineages were introduced in different moments in Goiás, Brazil, using distinct routes, likely originated from the Caribbean. Lineage I was first introduced coming from Rio de Janeiro (2007–2012), followed by the introduction from Argentina (2010 — 2013). Lineage II was introduced in a single moment from Rio de Janeiro and this clade has existed since 2007–2010. The different viral introduction events demonstrate the viral dispersion process with neighboring regions, which is essential for the maintenance of outbreaks and introduction of new emerging viruses. In conclusion, obtained data reveals the importance of continuous molecular surveillance of this virus in different regions, providing a better understanding of DENV-1 circulation, considering the evolutionary and virus spread patterns.

1. Introduction

Dengue viruses (DENVs) are the world's most important mosquito borne viral pathogens for humans in terms of morbidity, mortality and economic impact (Messina et al., 2014). DENV is transmitted to humans during feeding of infected *Aedes* mosquitoes, mainly *Aedes aegypti*, which is widely distributed around the tropical and subtropical regions of the world (Gubler, 1998). DENV is an enveloped virus of the genus *Flavivirus*, family *Flaviviridae*, and classified in four phylogenetically and antigenically distinct serotypes (DENV-1-4) that cause outbreaks among humans.

The genome is a single-stranded, positive-sense RNA of approximately 11 kb, which contains a single open reading frame encoding three structural [capsid (C), membrane (M) and envelope (E)] and seven non-structural (NS1, NS2A, NS2B, NS3, NS4A, NS4B and NS5) proteins (Chambers et al., 1990). Based on genetic diversity and geographical distribution, distinct groups have been also identified within each serotype, defined as genotypes, which may also differ in viral virulence/transmissibility (Cologna et al., 2005).

For DENV-1 five distinct genotypes are recognized, genotypes I–V, (Allicock et al., 2012; Cologna et al., 2005; Costa et al., 2012). The genotypes I, IV and V are contemporary, while genotypes II and III are extinct (Mendez et al., 2010; Mizuno et al., 2012; Raghwani et al., 2011; Villabona-Arenas and Zanotto, 2013). Moreover, phylogenetic studies have revealed viruses clustered in clades within each genotype, characterizing distinct lineages (Holmes and Twiddy, 2003; Mendez et al., 2010; Santos et al., 2011; Weaver and Vasilakis, 2009).

According to the World Health Organization (WHO) DENV is endemic in more than 100 countries and it is estimated that 50 million cases occur annually worldwide (WHO, 2009). From 2011 to 2014 the National Surveillance system reported in Brazil more than 4.38 million suspected cases of dengue representing 61.7% of dengue cases in the Americas. In 2013, the highest number of dengue cases was notified when compared to the previous years. Over 1.4 million suspected dengue cases, of which 6969 with severe manifestations and 545 deaths

* Corresponding author at: Departamento de Microbiologia, Instituto de Patologia Tropical e Saúde Pública, Universidade Federal de Goiás, Rua 235, s/n, Setor Universitário, CEP: 74605-050 Goiânia, Goiás, Brazil.
E-mail address: fabiolasf@gmail.com (F.S. Fiaccadori).

http://dx.doi.org/10.1016/j.meegid.2016.05.021
1567-1348/

(PAHO, 2013), with concurrent circulation of four serotypes. The Central West and Southeast regions were leaders in cases notifications in Brazil, and the state of Goiás reported over 160 thousand cases in 2013 (SES, 2013).

Phylogenetic and phylogeographic analyses based on molecular data have been used to analyze the genetic diversity of DENV, to characterize DENV serotypes/genotypes/lineages, and to support previous molecular epidemiological studies (Allicock et al., 2012; Costa et al., 2012; Mendez et al., 2010; Mizuno et al., 2012; Raghwani et al., 2011; Santos et al., 2011; Villabona-Arenas and Zanotto, 2013). Despite the relevance of dengue virus, the phylodynamics of DENV-1 in Central-West region were still unknown. The present study describes the genetic characterization and the spatiotemporal patterns of DENV-1 spread in the state of Goiás, during the 2013 outbreak.

2. Materials and methods

2.1. Viral strains

The strains analyzed in this study were obtained from 278 patients with clinical suspicion of dengue infection in 2013 during the epidemic period in Goiás, Brazil. These samples were previously serotyped (data not published) using the protocol described by Lanciotti et al. (1992). Sixteen DENV-1 positive samples were select. This study was approved by the Hospital Materno Infantil — Ethical Committee in Research (HMI-CEP 17/12).

2.2. Sequencing of E gene region

Viral RNA was extracted using the TRIzol® reagent (Life Technologies) following the manufacturer's instructions. Reverse transcription was performed with 14 μl of RNA in a 50 μl reaction mix containing: 0.4 ng/μl random hexamers (Invitrogen); 2 mM dNTPs (Invitrogen); 1 mM of $MgCl_2$ (Invitrogen); 80 U of Moloney Murine Leukemia Virus Reverse Transcriptase (*M-MLV*) (Invitrogen); 8 U of RNAseOUT™ (Invitrogen); 2 mM Dithiothreitol (DTT) (Invitrogen); buffer 5× – $MgCl_2$ (Invitrogen) and Nuclease-free water. The reaction was carried out in a thermal cycler (Esco PCR Thermal Cyclers) as follows: 42 °C for 5 min followed by 37 °C for 30 min.

PCR amplification reaction of the complete envelope (E) gene (1485 nt) was performed using 3 μl of cDNA, GoTaq® Colorless Master Mix 2× (Promega Corporation), and Nuclease-Free Water, with three primers pairs described to DENV-1 by Zheng et al. (2006). Amplification was carried out as follows: 40 cycles at 95 °C for 30 s, 60 °C for 40 s, and 72 °C for 60 s, followed by a final extension at 72 °C for 10 min. The PCR products were precipitated with 65% isopropanol, washed with 60% ethanol and diluted in 20 μl Nuclease-Free Water for the PCR products purification. Sequencing reactions were performed with the BigDye terminator v.3.1 cycle sequencing kit (Applied Biosystems) using the three primers pairs in the Genetic analyzer automated sequencer "Applied Biosystems PRISM" 3130 (Applied Biosystems).

2.3. Envelope gene assembly and accession numbers

Chromatograms were analyzed in Codon Code Aligner 3.7.1 (Sequence Assembly and Alignment Software — CodonCode Corporation) with a Phred quality score of 20 as cut-off for trimming of low-quality sequences. The identification of equal sequences was inferred using the DNAsp v.5 program (DNA Sequence Polymorphism) (Librado and Rozas, 2009). The sequences identified in this study were deposited in GenBank (accession nos. KP858105–KP858119).

2.4. Data sets

The DENV-1 E gene sequences from Brazil and others countries in the world were recovered for all known genotypes and used in our analysis. Duplicate threads and uninformative sequences (sequences that did not have completed information about geographic origin and also sequences that had high nucleotide identity, and that were characterized on the same temporal and spatial scale) were not used in the final analysis. This resulted in a final data set of 91 DENV-1 E gene sequences (1485 nt long) from: Asia ($n = 21$), North America ($n = 4$), Central America ($n = 15$) and South America ($n = 51$) covering a total of 24 countries (Table S1). All sequences are presented in the format: gi number/country/year of isolation in the phylogenetic trees. Samples from Brazil are identified as gi number/BR-state/year of isolation. All recovered sequences were aligned using Clustal X2 (Thompson et al., 1997) and edited using jalview (Clamp et al., 2004). Only aligned and edited sequences were used in the phylogenetic analysis. The amino acid (aa) substitutions analysis was performed using MEGA 6 (Tamura et al., 2013).

2.5. Evolutionary reconstruction of DENV-1 dispersal

Bayesian Inference (BI) analysis were conducted by using general time-reversible with gamma-distributed rate variation substitution model and a proportion of invariant sites (GTR + I + G), as described by Akaike's information criterion (AICc) in jModelTest 0.1 (Darriba et al., 2012).

To estimate the time to the most recent common ancestor (tMRCA) and to trace the geographic flow of DENV-1 over time, the Markov Chain Monte Carlo (MCMC) algorithm in the BEAST package v.1.8.2 was used (Drummond and Rambaut, 2007). The calibration point was the date of isolation of each sample. In addition, information on the specific local isolation was assigned for each sequence, and a discrete phylogeographic analysis was conducted. Runs were performed using the Bayesian skyline as the tree prior under the uncorrelated lognormal relaxed molecular clock, using the estimated rate of 6.56E − 4 substitution/site/year, as previously described (Villabona-Arenas and Zanotto, 2013). The evolutionary analysis were run for 100 million steps and the trees were sampled every 10,000 states. Convergence of the MCMC chains were inspected using TRACER v.1.6 (http://tree.bio.ed.ac.uk). Posterior trees were summarized discarding the first 20% of the sampled trees and choosing the Maximum Clade Credibility (MCC) were summarized using TreeAnnotator v.1.8.2. The final tree was then visualized and plotted using FigTree v.1.4.2 (http://tree.bio.ed.ac.uk). SPREAD v.1.0.6 application was used to visualize and convert the estimated divergence times. Spatial estimates were annotated in the MCC trees to a keyhole language file. All evolutionary parameters were reported as posterior means along with their 95% Bayesian credibility intervals.

3. Results

A total of 16 confirmed DENV-1 samples were sequenced and aligned with DENV-1 E gene sequences from GenBank resulting in a trivial alignment, considering that no indels were identified during sequence alignment.

Identity analysis of the sequences obtained in this work (1485 nucleotides) showed percentages identities ranging from 95.29% to 100%. Data demonstrated two equal sequences (100% identical), being represented by a single sequence in the phylogenetic analysis. Amino acid substitutions were observed in 22 positions, with 14 amino acid substitutions among the lineages I and II, including 10 substitutions considered non-conservative and four conservative (Table S2).

Bayesian Inference analysis comparing all the sequences produced a phylogenetic tree with the Brazilian sequences distributed in a monophyletic group, characterized as genotype V (America/Africa). The DENV-1 genotype V isolates circulating in 2013 in Goiás were clasterized in two distinct branches (Fig. 1), featuring the co-circulation of two genetically distinct lineages in spatial/temporal scale. Lineage I was associated with DENV-1 sequences isolated in the southeast (Rio de Janeiro, São Paulo and Espírito Santo) northeast (Pernambuco,