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Phylogenetic analysis of *Dengue virus* 1 isolated from South Minas Gerais, Brazil

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ABSTRACT

Dengue is a major worldwide public health problem, especially in the tropical and subtropical regions of the world. Primary infection with a single *Dengue virus* serotype causes a mild, self-limiting febrile illness called dengue fever. However, a subset of patients who experience secondary infection with a different serotype can progress to a more severe form of the disease, called dengue hemorrhagic fever. The four *Dengue virus* serotypes (1–4) are antigenically and genetically distinct and each serotype is composed of multiple genotypes. In this study we isolated one *Dengue virus* 1 serotype, named BR/Alfenas/2012, from a patient with dengue hemorrhagic fever in Alfenas, South Minas Gerais, Brazil and molecular identification was performed based on the analysis of NS5 gene. Swiss mice were infected with this isolate to verify its potential to induce histopathological alterations characteristic of dengue. Liver histopathological analysis of infected animals showed the presence of inflammatory infiltrates, hepatic steatosis, as well as edema, hemorrhage and necrosis focal points. Phylogenetic and evolutionary analyses based on the envelope gene provided evidence that the isolate BR/Alfenas/2012 belongs to genotype V, lineage I and it is probably derived from isolates of Rio de Janeiro, Brazil. The isolate BR/Alfenas/2012 showed two unique amino acids substitutions (SER222THRE and PHE306SER) when compared to other Brazilian isolates from the same genotype/lineage. Molecular models were generated for the envelope protein indicating that the amino acid alteration PHE 306 SER could contribute to a different folding in

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this region located within the domain III. Further genetic and animal model studies using BR/Alfenas/2012 and other isolates belonging to the same lineage/genotype could help determine the relation of these genetic alterations and dengue hemorrhagic fever in a susceptible population.

Introduction

Dengue virus (DENV) is an arbovirus transmitted to humans through the bite of infected *Aedes aegypti* mosquitoes. Dengue disease is endemic in several countries of Africa, the Americas, Mediterranean, Southeast Asia and the Western Pacific and almost half of the world's population live in risk areas for dengue.[1] DENV infects 50–100 million people each year and among the infected patients 500,000 are at risk to develop the more severe diseases, such as Dengue Hemorrhagic Fever (DHF).[2,3] DENV is an enveloped virus that belongs to the family *Flaviviridae*, genus *Flavivirus*. The viral genome encodes a single open reading frame which, when translated, produces a polyprotein that is processed into three structural proteins (capsid, membrane and envelope proteins) and seven non-structural proteins (NS1, NS2A, NS2B, NS3, NS4A, NS4B, NS5).

The phylogenetic analysis of DENV isolates provides insights into the evolutionary and the migratory processes of DENV, thus contributing to a better understanding of the epidemiology of the disease.[4–6] In Brazil, the circulation of all four DENV serotypes has been reported and several studies have revealed a substantial genetic diversity among Brazilian DENV serotypes/genotypes. One DENV-1 outbreak was reported in Northern region of Brazil, in 1981, but it only established wider circulation in the country a few years later. DENV-1 was probably reintroduced into Brazil in 1984–1985[7] and in 1986 an outbreak was reported in the Southeast region. Subsequently, DENV-1 spread throughout the country. In the following years, the other 3 serotypes (DENV-2, DENV-3 and DENV-4) were also introduced into Brazil.[8–10] The phylogenetic studies of Brazilian DENV-1 isolates revealed the circulation of different lineages of genotype V[7,11,12] introduced into the country in different times, probably from different countries, leading to lineage replacement or to the co-circulation of different DENV-1 lineages.[7]

The Minas Gerais State (Southeast region of Brazil) has been gone through recurrent epidemics of dengue. DENV was first described in Minas Gerais in 1987, and since 1996, regular outbreaks have caused considerable illness in the state. During 1987–2010 the viral isolation data showed the presence of DENV-1, DENV-2, and DENV-3 in Minas Gerais and only in 2011 was DENV-4 reported in the state. Beside the circulation of all 4 serotypes in the Minas Gerais State, DENV-1 continued to be the most frequent serotype observed in 2011 and 2012.[13] Despite the importance of the disease for the region, there have not been many studies that analyze the genetic variability of DENV isolates in this region. Therefore, this work aims to perform a molecular and phylogenetic analysis of a DENV-1 strain isolated in southern Minas Gerais state, Brazil.

Material and methods

Strain and sample preparation

The strain BR/Alfenas/2012 was obtained from a serum sample derived from a patient with dengue hemorragic fever, in 2012, in Alfenas, Minas Gerais State, Brazil. The patient presented skin hemorragic manifestations and laboratory findings demonstrated the increase in hematocrit concurrent with rapid decrease in platelet count. Serological tests confirmed the presence of anti-Dengue IgM and IgG (Panbio Dengue Duo IgM and IgG Capture ELISA, Alere, Australia) confirming thus, the secondary infection of this patient. Blood was collected seven days after the onset of the first symptoms upon authorization of the patient. This study was carried out by following the rules and laws that govern the use of human and animal material (approved protocol 08410912.9.0000.5142).

Cells and virus isolation

Aedes albopictus C6/36 cells were propagated in Leibovitz L15 medium (Cultilab, Brazil) supplemented with 10% fetal calf serum (Cultilab, Brazil) at 28 °C and then used for virus isolation. For virus isolation, a 50-μL sample of serum was incubated with C6/36 cells supplemented with 2% fetal calf serum. Infected C6/36 cells showing typical cytopathic effect were harvested, and the tissue culture supernatants were used for viral RNA extraction QiAmp Viral RNA kit (QIAGEN, USA). Uninfected cells were used as negative controls.

RT-PCR and sequencing of NS5 and Envelope gene

First reverse transcription and amplification was performed using universal primers targeting all three flavivirus subgroups as previously described.[14] The region amplified encoded part of the methyltransferase and most of the RNA-dependent-RNA-polymerase domain of NS5 and, given its conserved pattern, it was chosen for flavivirus detection and identification. Second, a reverse transcription and amplification of the full envelope gene was performed using the protocol previously described.[7] The envelope gene was chosen for phylogenetic and evolutionary analyses since it is a more variable gene that possesses a robust phylogenetic signal. All amplicons were sequenced using the Big Dye chemistry (USA) on ABI3730xl DNA sequencers according to Applied Biosystems protocols.