

January 2013

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Recommended Citation

Singhal, Nidhi and Bhandarkar, Manoj (2013) "Computational Analysis of Dengue and Chikungunya Viral Gene Sequences To Elucidate Their Transmission and/or Symptoms Similarity," *International Journal of Pharmacology and Pharmaceutical Technology*. Vol. 1 : Iss. 1 , Article 5.

DOI: 10.47893/IJPPT.2013.1002

Available at: <https://www.interscience.in/ijpjt/vol1/iss1/5>

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Computational Analysis of Dengue and Chikungunya Viral Gene Sequences To Elucidate Their Transmission and/or Symptoms Similarity

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Abstract - Dengue (DENV) and Chikungunya (CHIKV) viruses are enveloped positive-strand RNA mosquito-borne viruses that have been disseminated worldwide. Both the viruses are dissimilar in many aspects like genome organization and expression, particle size and taxonomical status except two similar aspects i.e. mode of transmission and the disease pathogenicity. Reports are indicating the co-infection of the both the viruses in a same patient. In view of similarities and/or dissimilarities between these two viruses, we intended to identify the existence of homology between any two gene sequences of which one from each virus. For this study we have analyzed all the structural and non structural nucleotide sequences of DENV type 1 (ACCESSION no. NC_001477) and CHIKV IND-06-Guj strain (ACCESSION no. JF272477) using the computational techniques. The results indicated that there is no homology between structural genes of both viruses. But analysis of the non-structural genes revealed that DENV NS4B and CHIKV nsp2 is showing the considerable homology. It is reported that DENV NS4B is involved in maintaining the replication balance in mosquito and human hosts. Hence we predict based on our results, CHIKV nsp2 which is showing homology with the DENV NS4B also serving same purpose in case of CHIKV. Further functional analysis of DENV NS4B and CHIKV nsp2 revealed the information that both have role in IFN inhibition and likely to have an important role in viral pathogenesis. Identification of common or shared epitopes on these polypeptides could lead to the development of an immunogen which could serve as vaccine candidate for both the viruses.

Keywords - Dengue (DENV), Chikungunya (CHIKV), Homology, NS4B, nsp2.

I. INTRODUCTION

Dengue fever and Chikungunya fever are mosquito-borne viral diseases distributed in most tropical and subtropical regions with an estimated 50–100 million human cases annually [1], [2], [3], [4] and [5]. In Asia, the CHIKV-affected areas overlap with DENV-endemic areas [6] and [7] and provide opportunities for mosquitoes to become infected with both viruses. Both share the same vectors, symptoms, and geographical distribution [8] and [9]. The flavivirus genome (DENV) is a single-stranded RNA of plus-sense polarity. The genomic RNA contains a 5' untranslated region (UTR), a single open reading frame, and a 3' UTR. The single open reading frame encodes a long polyprotein that is processed by viral and host proteases into 10 mature viral proteins. Three structural proteins (Capsid [C], premembrane [prM], and envelope [E]) are components of virus particles. Seven nonstructural proteins (NS1, NS2A, NS2B, NS3, NS4A, NS4B, and NS5) are responsible for viral replication [10]. Chikungunya virus (CHIKV) is an envelope, single-stranded, positive-sense RNA virus that belongs

to the family Togaviridae, genus Alphavirus [11], [12], [13] and [14].

It has long been recognized that computational methods and resources has the potential to accelerate biological and immunological research. Sequencing of the flaviviridae and togaviridae virus family and other model organism genomes has produced increasingly large volumes of data relevant to immunology research. At the same time, huge amounts of functional, clinical and epidemiologic data are useful for the researchers to understand the disease pathogenesis and development of target drugs. In this study, we have selected tools for phylogenetic and sequence analysis of structural and nonstructural genes of dengue virus against the structural and nonstructural genes of CHIKV. This study helps in contributing to find the similarity between them which could help in the finding of epitope same for both of the virus for the development of vaccine.

II. MATERIAL AND METHODS

All computational analysis was carried out on a Windows XP platform running on a Compaq PC with an Intel Core 2 Duo processor and 2 GB of RAM.

A. Sequence Retrieval

The genome of Chikungunya virus (IND-06-Guj) has 11829 nucleotides and contains five structural protein, namely core (783), E3 (192), 6k (183), E2 (1269), E1 (1320) and four nonstructural protein, namely nsP1 (1605), nsP2 (2394), nsP3 (1590), and nsP4 (1836) (Table.1). The nsP2 protease is an essential protein whose proteolytic activity is critical for virus replication.

The genome of dengue virus (type 1) has 10735 nucleotides and contains three structural proteins, namely capsid (300), envelope (1485), glycoprotein (225) and seven nonstructural proteins, namely NS1 (1065), NS2A (654), NS2B (390), NS3 (1858), NS4A (380), NS4B (746), NS5 (2696) (Table.2). All nucleotide sequence of DENV was (ACCESSION no. NC_001477) downloaded from GenBank at National Center for Biotechnological Information (NCBI) and CHIKV (ACCESSION no. JF272477) nucleotide sequence was collected from the EMBL-Bank Database. The DNA sequences of all genes of DENV-1 and CHIKV were used to identify the similar sequence between them.

| Sl. No. | CHIKV (component) | Nucleotide no. | Source |
|---------|-------------------|----------------|-----------|
| 1 | Core Protein | 783 | EMBL-Bank |
| 2 | E3 | 192 | EMBL-Bank |
| 3 | 6k | 183 | EMBL-Bank |
| 4 | E2 | 1269 | EMBL-Bank |
| 5 | E1 | 1320 | EMBL-Bank |
| 6 | nsP1 | 1605 | EMBL-Bank |
| 7 | nsP2 | 2394 | EMBL-Bank |
| 8 | nsP3 | 1590 | EMBL-Bank |
| 9 | nsP4 | 1836 | EMBL-Bank |

Table1: CHIKV structural and nonstructural genes with nucleotide no. and source.

| Sr. No. | DENV (component) | Nucleotide no. | Source |
|---------|-----------------------|----------------|--------|
| 1 | Capsid Protein | 300 | NCBI |
| 2 | Membrane glycoprotein | 1485 | NCBI |
| 3 | Envelope protein | 225 | NCBI |
| 4 | NS1 | 1065 | NCBI |
| 5 | NS2A | 654 | NCBI |
| 6 | NS2B | 390 | NCBI |
| 7 | NS3 | 1858 | NCBI |
| 8 | NS4A | 380 | NCBI |
| 9 | NS4B | 746 | NCBI |
| 10 | NS5 | 2696 | NCBI |

Table2: DENV structural and nonstructural genes with nucleotide no. and source.

B. Phylogenetic analysis

Phylogenetic analysis is a method that is used to predict the relationship/identity between the genome sequences with an accuracy that is useful for the further analysis of genomes. The phylogenetic analysis was performed separately for structural and nonstructural nucleotide sequences of DENV and CHIKV viruses using ClustalW (www.ebi.ac.uk/Tools/clustalw2/index.html) and also for nonstructural nucleotide sequence of DENV and HCV. ClustalW2 is a general purpose multiple sequence alignment program for DNA or proteins.

C. Pairwise analysis by use of local alignments

A similarity search for DENV NS4B and CHIKV nsP2 nucleotide sequence was performed. Pairwise comparisons of the DENV-1 and CHIKV database were done by local alignment by using the algorithm of Smith-Waterman algorithm, implemented by a program from EMBOSS, the European Molecular Biology Open Software Suite and by LALIGN. LALIGN finds internal duplications by calculating non-intersecting local alignments of protein or DNA sequences. The two Sequences were aligned, analyzed, subjected to homology search by Lalign (http://www.ch.embnet.org/software/LALIGN_form.html) and EMBOSS (<http://www.ebi.ac.uk/Tools/emboss/align/>) server.

III. RESULTS AND DISCUSSION

A. Identification of homology of Structural and Nonstructural gene of DENV and CHIKV using multiple sequence analysis (ClustalW)

The multiple sequence alignment using ClustalW is a good method to find out the relationship among the genes. The structural and nonstructural nucleotide

sequences of DENV and CHIKV were used to predict the homology in between both of the virus genome. According to the multiple sequence analysis of structural genes of DENV and CHIKV showed a non significant similarity when analyzed by ClustalW phylogram (Fig. 1). Analysis of DENV and CHIKV nonstructural genes by ClustalW showed a significant similarity. Fig. 2 is showing the phylogram of this study. As indicated in Fig. 2 showed that DENV NS4B and CHIKV nsP2 have the same origin in phylogenetic tree i.e. both have identity and also both have the same function as well.

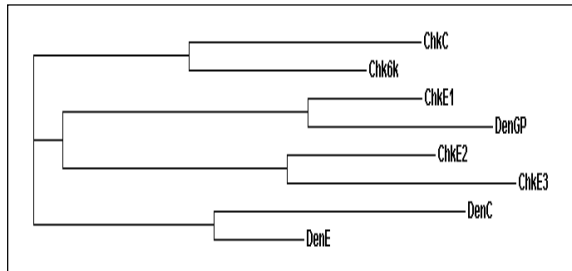


FIG.1. Phylogenetic tree between DENV-1 and CHIKV generated by ClustalW2 method based on the nucleotide sequence of all structural genes and Data of DENV-1 and CHIKV nucleotide sequence were retrieved from the National Centre for Biotechnology NCBI database (www.ncbi.nlm.nih.gov) and EMBL-Bank (<http://www.ebi.ac.uk/emb/>) respectively. Phylogenetic tree showing same origin in between DENV M(Membrane Glycoprotein) and CHIKV E1 protein but distance is more.

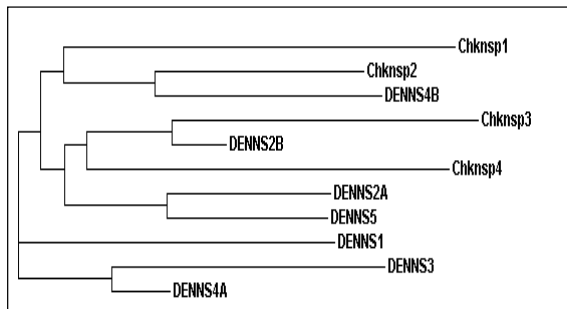


FIG.2. Phylogenetic tree between DENV-1 and CHIKV generated by ClustalW2 method based on the nucleotide sequence of all nonstructural genes and Data of DENV-1 and CHIKV nucleotide sequence were retrieved from the National Centre for Biotechnology NCBI database (www.ncbi.nlm.nih.gov) and EMBL-Bank (<http://www.ebi.ac.uk/emb/>) respectively. Phylogenetic tree showing same origin in between two sets of gene between DENV and CHIKV, first set is in between DENV NS4B and CHIKV nsP2 and second set is in between of DENV NS2B and CHIKV nsP3 protein

but distance between of DENV NS2B and CHIKV nsP3 is more which is not considerable.

B. Identification of homology of Nonstructural gene of CHIKV and HCV using multiple sequence analysis (ClustalW) as a positive control

We also did the multiple sequence alignment of CHIKV and HCV nonstructural genes using ClustalW program. It's didn't showed any significant similarity between any of two gene of CHIKV and HCV (Fig. 3). HCV is not a mosquito borne disease as CHIKV so it is not showing any functional similarity also.

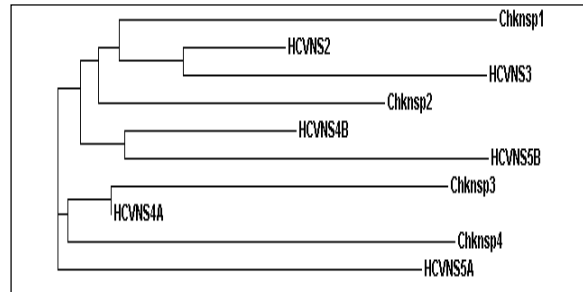


FIG.3. Phylogenetic tree between HCV and CHIKV generated by ClustalW2 method based on the nucleotide sequence of all nonstructural genes and Data of HCV and CHIKV nucleotide sequence were retrieved from the National Centre for Biotechnology NCBI database (www.ncbi.nlm.nih.gov).

C. Identification of homology between DENV NS4B and CHIKV nsP2 gene using Two sequence analysis

In order to corroborate our ClustalW result of nonstructural gene, two sequence analysis was performed. To elucidate the identity between DENV nonstructural gene NS4B and CHIKV nonstructural gene nsP2 we carried out two sequence analysis using various tools like Lalign, EMBOSS to find out percentage identity between them.

D. Lalign analysis

To determine the identity of both of the gene we investigated DENV NS4B and CHIKV nsP2 gene with further Lalign server. Lalign analysis showed a 66.67 % identity in 139 nucleotides in between of NS4B and nsP2 gene (Fig. 4).

E. EMBOSS analysis:

To confirm the identity between DENV NS4B and CHIKV nsP2 gene we further went for the EMBOSS analysis with a gap penalty of 10 and extend penalty of 0.5. The results of Local alignment with EMBOSS revealed the identity in between both of the gene with 46.6% similarity. The two sequence analysis of two nonstructural genes of DENV and CHIKV, NS4B and

nsP2 respectively showed a significant homology (Fig. 5).

F. Functional analysis

Functional analysis of NS4B and nsP2 suggests that both of the gene having the same function. Both of the genes are involved in IFN signaling inhibition and also likely to have an important role in viral Pathogenesis. DENV NS4B gene has a role in replication of mosquito and mammalian system.

IV. CONCLUSION

Identification of common or shared epitopes of DENV NS4B and CHIKV nsp2 gene could lead to the development of an immunogen which could serve as vaccine candidate for both the viruses.

V. ACKNOWLEDGEMENTS

The authors like to thank the Department of Biotechnology, Madhav Institute of Science And Technology, Gwalior, India for its support and providing the facilities.

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