A Conserved Region Common to Chikungunya (ChikV), Dengue (DenV) and Zika Viruses (ZikV): Potential as a Tool for Simultaneous Diagnosis and Therapeutics of the Three Viruses [Short Communication]

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Abstract There is an increasing need to develop strategies for simultaneous detection of Chikungunya (ChikV), Dengue (DenV) and Zika Virus (ZikV) owing to their shared transmission ecology. Towards this aim, nine reference sequences of ChikV, DenV (1 — 4) and ZikV were aligned using ClustalW Omega Software available at the European Bioinformatics Institute (EBI) website. Internet search engines like Google (Scholar), PubMed, JSTOR, and ProQuest Central were used for literature search. Also, Conserved Domain Database (CDD) (NCBI) and ZikaVR database maintained by IMTECH, Chandigarh, India, formed constant references. One conserved region, 21 amino acids (aa) in length, common to the three viruses mapping to the Core protein of ChikV (aa 211 — 231); and the NS3 proteins of DenV (1 — 4) (aa 1608 – 1628) and ZikV (aa 1631/35 — 1651/55) has been identified by the current author in this study. This region is the part of a protease in all the three viruses studied. Thus, this conserved region can form one diagnostic tool/probe for simultaneous detection of ChikV, DenV and ZikV. Additionally, as this region is conserved, it may form one therapeutic target.

Keywords ChikV, DenV, ZikV, Simultaneous, Diagnosis, Conserved, Core, NS3

1. Introduction

Recent observations of the World Health Organization (WHO) indicate that the disease dynamics of Zika Virus (ZikV) and Dengue Virus (DenV) seem to be working in tandem. Note that after the year 2016, when there was an epidemic with ZikV, the number of DenV cases were relatively down [1, 2]. Also, ZikV, DenV and a third virus, the Chikungunya Virus (ChikV), share epidemiological parameters like transmission ecology [2, 3]. India is recognised as an endemic country to the viruses in context [4]. Furthermore, there are reports of the three viruses being isolated from a single mosquito and reports of natural co-infections with any two or all the three viruses in humans [5, 6, 7]. Additionally, the symptoms of many pathogenic infections overlap with those of the infections caused by either ChikV, DenV or ZikV. Thus, there is an urgent need for developing strategies for simultaneous diagnosis of the three viruses. Such a work would initially involve identification of regions that are common and conserved to the three viruses being studied for the present.

2. Materials and Methods

2.1. Literature Search

Literature search was performed using internet search engines like Google (Scholar), PubMed, JSTOR, and ProQuest Central (author’s personal membership at the British Council Library, Hyderabad). In addition to this, Conserved Domain Database (CDD) at the National Center for Biotecnology Information (NCBI) and ZikaVR database maintained by IMTECH, Chandigarh, India, formed constant references.

2.2. Reference Sequences

Characteristics of ChikV, DenV and ZikV are summarized as table 1.
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Table 1. Characteristics of the three viruses considered in the present study

| Characteristic | Chikungunya Virus (ChikV) | Dengue Virus (DenV) | Zika Virus (ZikV) |
|---------------|---------------------------|---------------------|------------------|
| Genus         | Alphavirus                | Flavivirus          | Flavivirus       |
| Family        | Togaviridae               | Flaviviridae        | Flaviviridae     |
| Natural Host  | Vertebrates               | humans, monkeys     | Vertebrates      |
| Vector / Reservoir | Aedes sp. (Mosquito) | Aedes sp. (Mosquito) | Aedes sp. (Mosquito) |
| Clinical Disease | Unsymptomatic rash, arthralgia, fever | Dengue (with or without symptoms) & Severe Dengue – characteristic high fever, rash | Zika fever (acute fever and rash) often self-limiting and asymptomatic |
| Nucleic Acid  | (+) ss RNA                | (+) ss RNA          | (+) ss RNA       |

Legend. RNA – Ribonucleic acid; ss – Single stranded; Other abbreviations carry their usual significance.

Table 2. Details of the reference sequences used in the present study

| Sl. No. | Virus                     | Protein | Accession Number | Length |
|---------|---------------------------|---------|------------------|--------|
| 1       | Chikungunya Virus (ChikV) | Structural | ABN04200      | 1248 aa |
| 2       | Chikungunya Virus (ChikV) | Structural | ATW74975       | 1248 aa |
| 3       | Chikungunya Virus (ChikV) | Structural | NP_690589     | 1248 aa |
| 4       | Dengue Virus 1 (DenV 1)   | Polypeptide | NP_659433       | 3392 aa |
| 5       | Dengue Virus 2 (DenV 2)   | Polypeptide | NP_056776       | 3391 aa |
| 6       | Dengue Virus 3 (DenV 3)   | Polypeptide | YP_001621843   | 3390 aa |
| 7       | Dengue Virus 4 (DenV 4)   | Polypeptide | NP_073286      | 3387 aa |
| 8       | Zika Virus (ZikV)         | Polypeptide | YP_002790881   | 3419 aa |
| 9       | Zika Virus (ZikV)         | Polypeptide | AWH65848       | 3423 aa |

Legend. aa – amino acid; Other abbreviations carry their usual significance.

Towards the present aim, nine reference sequences of ChikV, DenV (1 — 4) and ZikV were considered and accessed from the NCBI-PubMed databases at https://www.ncbi.nlm.nih.gov/pubmed/. Details of the reference sequences used in the present study are given as Table 2.

2.3. Alignment of the Sequences

The nine reference sequences were aligned using ClustalW Omega Software available at the European Bioinformatics Institute (EBI) website (https://www.ebi.ac.uk/). Default parameters were used for aligning the sequences.

2.4. Identification of Conserved Region Common to the Three Viruses Being Studied

From the alignment output, a conserved region of 21 amino acids in length was identified manually without the help of any software. Figure 1 shows the conserved region in the aligned sequences. Figure 2 shows the identification of hypervariable regions (HVR) in the three viruses.
Figure 1. Conserved region common to ChikV, DenV and ZikV identified in the present study (box)
3. Results and Discussion

Table 3 presents the details of the one conserved region, 21 amino acids (aa) in length, common to the three viruses mapping to the Core protein of ChikV (aa 211 — 231); and the NS3 proteins of DenV (1 — 4) (aa 1608 — 1628) and ZikV (aa 1631/35 — 1651/55) identified by the author in this study. This region is the part of a protease in all the three viruses studied. Thereby this region is of significance to the three viruses in context. Thus, this region not only forms one diagnostic tool but a potential therapeutic target. A simple BLAST search at NCBI done with the conserved sequences mentioned in table 3 shows relative conservation across Alphavirus species of which ChikV is a member (data not shown).

Goh et al [8] report the reactivity of monoclonal antibodies against 27 synthetic peptides of ChikV including the conserved region identified in this study. However, Goh et al could not find any reactivity against this conserved region. In addition to this, Goh et al report the presence of one potential phosphorylation site in the identified region. It is worth mentioning at this stage that phosphorylation is a post translational modification and forms one potential therapeutic target in many viruses [9]. Goh et al in their publication also mention that the conserved region GDSG (initial four amino acids of the conserved region identified in the present study) might be involved as the catalytic triad residues involved in the auto-proteolytic function of the core protein. The ZikaVR database developed by Gupta et al [10] lists a portion of the conserved region in the Zika virus as a potential MHC-I epitope.

A significant number of publications discuss the need for simultaneous detection of ChikV, DenV and ZikV. Notable among these is the publication of Lura et al [11] who describe the effectiveness of the commercially available kits. Despite this, none of the publications mention that the
identified region shown in figure 1 and table 3 is conserved among all the three viruses in context, which this publication does. A hypervariable region (HVR) is characteristic of RNA viruses. It may be noted that the three viruses are RNA viruses. In the present study it was also observed that all the three viruses exhibit variability in their sequences (Figure 2; data of individual virus strains comparisons not shown). Given these observations, the importance of identifying a conserved region in ChikV, DenV and ZikV gains momentum.

4. Conclusions

To the best of the authors’ knowledge, this is the first publication identifying a conserved region that is common to ChikV, DenV and ZikV. This conserved region can form one diagnostic tool/probe for simultaneous detection of ChikV, DenV and ZikV. Additionally, as this region contains one potential phosphorylation site, it may form one therapeutic target against ChikV, DenV and ZikV.

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