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 (IMTECH, C'garh) 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 Biotechnology 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.

Table 1. Characteristics of the three viruses considered in the present study

Virus→ 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. N°.	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)	Polyprotein	NP_659433	3392 aa
5	Dengue Virus 2 (DenV 2)	Polyprotein	NP_056776	3391 aa
6	Dengue Virus 3 (DenV 3)	Polyprotein	YP_001621843	3390 aa
7	Dengue Virus 4 (DenV 4)	Polyprotein	NP_073286	3387 aa
8	Zika Virus (ZikV)	Polyprotein	YP_002790881	3419 aa
9	Zika Virus (ZikV)	Polyprotein	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.

Input form	Web services	Help & Documentation	Bioinformatics Tools FAQ
ABN04200.1	-----YACLVGDKVMKPAHVKGITDNADLAK--LAFKRSSKYDLEC-AQ-		166
ATW74975.1	-----YACLVGDKVMKPAHVKGITDNADLAK--LAFKRSSKYDLEC-AQ-		166
NP_690589.2	-----YACLVGDKVMKPAHVKGITDNADLAK--LAFKRSSKYDLEC-AQ-		166
YP_002790881.1	MQEGVFHTMWHVTKGAAALRSSEGRLDPYMGDVK---QDLVSYCGPWKLDAAWDGLSEVQL		1595
AWH65848.1	MQEGVFHTMWHVTKGSAALRSSEGRLDPYMGDVK---QDLVSYCGPWKLDAAWDGHSEVQL		1599
NP_073286.1	HMEGVFHTMWHVTRGSVICHETGRLEPSWADVR---NDMISYGGGWRLGDKWDKEEDVQV		1571
NP_056776.2	YKEGTFTMWHVTRGAVLMHKGKRIEPSWADVK---KDLISYGGGWRLGEGWKEGEEVQV		1572
NP_059433.1	FQEGVFHTMWHVTRGAVLMYQGRLEPSWASVK---KDLISYGGGWRFQGSWNAEGEEVQV		1572
YP_001621843.1	QKEGVFHTMWHVTRGAVLTHNGKRLLEPNMASVK---KDLISYGGGWRLSAQWQKEEEVQV		1570
ABN04200.1	IPVHMKSDASKFTHKEPEGYYNHHGAVQYSGGRFTIPTGAGKP		226
ATW74975.1	IPVHMKSDASKFTHKEPEGYYNHHGAVQYSGGRFTIPTGAGKP		226
NP_690589.2	IPVHMKSDASKFTHKEPEGYYNHHGAVQYSGGRFTIPTGAGKP		226
YP_002790881.1	LAVPP-GERAR-----NIQTLPGIFKTKDGDIGAVLDYPAGTSGSPILDKCGRVIG		1646
AWH65848.1	LAVPP-GERAR-----NIQTLPGIFKTKDGDIGAVLDYPAGTSGSPILDKCGRVIG		1650
NP_073286.1	LAIEP-GKNPK-----HVQTKPGLFKLTGEGAVTLDFKPGTSGSPIINRKGKVG		1622
NP_056776.2	LALIEP-GKNPK-----AVQTKPGLFKTNAGTIGAVSLDFSPGTSGSPIIDKKGKVG		1623
NP_059433.1	IAVEP-GKNPK-----NVQTPAGTFKTPGEVGAIALDFKPGTSGSPIVNRGKIVG		1623
YP_001621843.1	IAVEP-GKNPK-----NFQTPGTFTTTGEIGAIALDFKPGTSGSPIINREGKIVG		1621
ABN04200.1	IVLGGANEGARTALSVVTWNKDIVTKITPEGAEWS--LAIPVMCLLANTTFPCSQPPCA		284
ATW74975.1	IVLGGANEGARTALSVVTWNKDIVTKITPEGAEWS--LAIPVMCLLANTTFPCSQPPCI		284
NP_690589.2	IVLGGANEGARTALSVVTWNKDIVTKITPEGAEWS--LAIPVMCLLANTTFPCSQPPCI		284
YP_002790881.1	LYGNGVVIKNGSYVSAITQGRREEETPVECFEPSMLKKKQLTVLDLHPGAGK---TRRVL		1703
AWH65848.1	LYGNGVVIKNGSYVSAITQGRREEETPVECFEPSMLKKKQLTVLDLHPGAGK---TRRVL		1707
NP_073286.1	LYGNGVTKSGDYVSAITQAERIGE-POYEVEDIFRKKRLTIMDLHPGAGK---TKRIL		1678
NP_056776.2	LYGNGVTRSGAYVSAIAQTEKSIE-DNPEIEDIFRKKRLTIMDLHPGAGK---TKRYL		1679
NP_059433.1	LYGNGVTTSGTYVSAIAQAKASQEGPLPEIEDEVFRKKRLTIMDLHPGSGK---TRRYL		1680
YP_001621843.1	LYGNGVTKNGGYVSGIAQTNAEPDGPTELEEMFKKKRLTIMDLHPGSGK---TRKYL		1678
ABN04200.1	PCCYEKEPEKTLRMLLEDNMSPGYQLLQASLTCSPPRQRRSIKDNFNVYKATRPYLAHC		344
ATW74975.1	PCCYEKEPEETLRMLLEDNMSPGYQLLQASLTCSPPRQRRSTKDNFNVYKATRPYLAHC		344
NP_690589.2	PCCYEKEPEETLRMLLEDNMSPGYQLLQASLTCSPPRQRRSTKDNFNVYKATRPYLAHC		344
YP_002790881.1	PEIVREAIKKRLR---TVILAPTRVV-----AAEMEALRGLPVRVMTTAVNVTHS		1751
AWH65848.1	PEIVREAIKRLR---TVILAPTRVV-----AAEMEALRGLPVRVMTTAVNVTHS		1755

Figure 1. Conserved region common to ChikV, DenV and ZikV identified in the present study (box)

Query_10006	381	WGNGCGLFGKGLVTCACFKQCLEPIEGKVQYE-NLKYTVIITVHTGDQHQ-----VGNET--QGVTAEE--ITPQASTT	449
Query_10007	380	WGNGCGLFGKGVVTCACFKSCSKITGNLVQIE-NLEYTVVVTVHNGDTHA-----VGNDSNHGVTAM--ITPRSPSV	450
Query_10008	391	WGNGCGLFGKGLVTCACFKTCKMTGKSIQPE-NLEYRIMLSVH-GSQHSGMI----GYET--DEDRAKVEVTPNSPRA	462
Query_10009	391	WGNGCGLFGKGLVTCACFACSKMTGKSIQPE-NLEYRIMLSVH-GSQHSGMIvndTGHET--DENRAKVEITPNSPRA	466
Query_10001	418	ITGTMGHFIL[7]TLTVGFTDGRKISHSCTHPFHDDPPVIGREKFHSRP[5]LPCSTYAQSTAATAEEIEVHMPDTP--	501
Query_10002	418	ITGTMGHFIL[7]TLTVGFTDSRKISHSCTHPFHDDPPVIGREKFHSRP[5]LPCSTYVQSNAAATAEEIEVHMPDTP--	501
Query_10003	418	ITGTMGHFIL[7]TLTVGFTDSRKISHSCTHPFHDDPPVIGREKFHSRP[5]LPCSTYVQSNAAATAEEIEVHMPDTP--	501
Query_10004	452	EIQLTDYGAL TLDSPRTGLDFNEMVLLTMKKKSHLVHKQWFLDLP LPWTSGASTSQETMNRQDLLVTFKTAHA	525
Query_10005	452	EAEITGYGTV TMECSPTGLDFNEMVLLQMNKAWLVHRQWFLDLP LPWLPGADTQGSNNIQQETLVTFKNPHA	525
Query_10006	450	EAILPEYGTI GLECSPTGLDFNEMILLTMKNKAMVHRQWFFDLP LPWASGATTETPTMNRKELLVTFKNPHA	523
Query_10007	451	EVKLPDYGEL TLDCEPRSGIDFNEMILMKKKKTWLVHKQWFLDLP LPWTAGADTSEVHNNYKERMVTFKVPHA	524
Query_10008	463	EATLGGFGSL GLDCEPRTGLDFSDLYLTMNNKHWLVHKEWFHDIP LPWHAADTGTPHNNKEALVEFKDAHA	536
Query_10009	467	EATLGGFGSL GLDCEPRTGLDFSDLYLTMNNKHWLVHKEWFHDIP LPWHAADTGTPHNNKEALVEFKDAHA	540
Query_10001	502	---DRTLSQQSGNVKITVNGRTvryKNCNGDSNEGLTtTDKVINNCKVDQCHAAVTNHKKWQYNSPLVPRNAELGDRKG	578
Query_10002	502	---DRTLSQQSGNVKITVNGRTvryKNCNGDSNEGLTtTDKVINNCKVDQCHAAVTNHKKWQYNSPLVPRNAELGDRKG	578
Query_10003	502	---DRTLSQQSGNVKITVNSQTvryKNCNGDSNEGLTtTDKVINNCKVDQCHAAVTNHKKWQYNSPLVPRNAELGDRKG	578
Query_10004	526	KKQEVVVLGSGEGAMHTALTGAT---EIQTSGTT-TIF-AGHLKCRLLKMDKLILKGMVYVMCTGSFKLEKEVAETQHGTV	600

Figure 2. Identification of the Hypervariable Region (HVR) in Chikungunya, Dengue (1 — 4) and Zika viruses.

Legend: Query_10001 = ABN04200.1 [Chikungunya virus]; Query_10002 = ATW74975.1 [Chikungunya virus]; Query_10003 = NP_690589.2 [Chikungunya virus]; Query_10004 = NP_059433.1 [Dengue virus 1]; Query_10005 = NP_056776.2 [Dengue virus 2]; Query_10006 = YP_001621843.1 [Dengue virus 3]; Query_10007 = NP_073286.1 [Dengue virus 4]; Query_10008 = YP_002790881.1 [Zika virus]; Query_10009 = AWH65848.1 [Zika virus]; Other abbreviations and symbols carry their usual significance. *Smaller black box* – Location of type specific hypervariable domain of Dengue virus strains as identified by Shiu *et al. J Gen Virol*, 1992; 73: 207 – 212. *Larger green box* – Location of the hypervariable region in the three viruses in context.

Table 3. Details of the conserved region identified in the present study

Sl. N°.	Virus	Sequence of the identified region*	Position	Potential glycosylation sites	Protein to which the sequence belongs	Reported domains in the PubMed
1	ChikV	GDSG RPIF DNKG RVVA IVLG G	211 – 231	None	Core	Peptidase_83
2	DenV	GTSG SPIL DKCG KVIG LYGN G	1608 – 1628	None	NS3	Serine protease
3	ZikV	GTSG SPIL DKCG RVIG LYGN G	1631/1635 – 1651/1655	None	NS3	NS3 Serine protease/part of a predicted MHC-1 binder/might be a potential drug target (ZikaVR db)

Legend. ChikV – Chikungunya Virus; DenV – Dengue Virus; db – database; ZikV – Zika virus; Other abbreviations carry their usual significance; *potential phosphorylation site bolded and shown in red colour. Phosphorylation site reported in ChikV by Goh *et al* [11] and by corollary in the other two viruses.

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 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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