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Comparative Molecular and Structural Characterisation of Chikungunya Virus Isolated Before and After 2006 Epidemics

K. Sangeetha[1], Indu Purushothaman[1] and S. Rajarajan[1*]

[1]Department of Microbiology and Biotechnology, Presidency College (Aut), Chepauk, Chennai, India.

Authors' contributions

This work was carried out in collaboration between all authors. Author SR designed the study and drafted the manuscript. Author KS performed the in vitro studies. Author IP performed the in silico analysis. All authors read and approved the final manuscript.

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ABSTRACT

Background: Chikungunya virus caused incapacitating infection during 2006 epidemics spanning 28 countries. The expansion of the disease and increased virulence resulted in neurological and ocular complications in the affected patients implicating changes in the structural and functional properties of the newly emerging strains of Chikungunya virus.
Objective: Hence the study was designed to understand the disparity between two strains isolated before and after 2006 epidemics by both *in vitro* and *in silico* approaches with respect to E1 gene.
Materials and Methods: Sequencing of E1 gene and phylogenetic analysis of the two strains were carried out followed by the determination of growth pattern. The impact of aminoacid substitutions on the structural properties of E1 protein between the strains of Chikungunya virus was identified by different Bioinformatic tools.
Results: Sequencing and Phylogenetic analysis revealed the two strains as Asian (isolated before 2006) and East Central South African (isolated after 2006). ECSA strain produced 1.5 fold $\log_{10}$ titre increased viral production than the Asian strain at the multiplicity of infection 1. Influence of aminoacid difference on the structure of E1 protein between two strains by Bioinformatic analysis

**Corresponding author: E-mail: rajarajan7101@gmail.com, drsrajarajan@gmail.com;*

had shown a change in the conformations by the loss of two intermolecular hydrogen bonds in 121 position in Asian strain and electrostatic differences in 211 positions between the two strains was also observed.

Conclusion: As the interacting aminoacid 121 and 211 position lies near the fusion loop hence the difference in aminoacid position between the two strains could better influence fusogenicity and stable trimer formation. This forms a preliminary insight on the impact of aminoacid substitutions on the structural properties of E1 protein between the strains of Chikungunya virus and further experimental investigations are warranted.

Keywords: Asian clade; east central South African clade; Chikungunya virus; fusogenicity.

1. INTRODUCTION

Mutation in RNA viruses occurs rapidly because of the high error frequency of viral RNA dependent RNA polymerases leading to adaptive mutation in host cells [1]. Mutation in Chikungunya virus has led to the emergence of new strains with higher degree of virulence and expansion into other countries [2]. So far Phylogenetically, Chikungunya virus has been classified into three different clades, primarily by geography into West African, Central / East African and Asian CHIKV [3,4]. But the epidemics after 2007 had led to the emergence of Indian Ocean lineage [IOL], a new lineage evolved from an existing ECSA enzootic genotype [5].

E1 protein plays a major role in the fusion of virus to the host cell receptors and it occurs through a p H –dependent endocytic pathway. Thus the amino acid and the structure of E1 protein highly influence the fusogenicity both in vector and host cells [6,7]. The change or substitution of amino acid from A226V mutation in E 1 protein had established the adaptability of Chikungunya virus in *Aedes albopictus* in addition to its rural vector, *Aedes aegypti* and had clearly demonstrated the role of aminoacid substitution in E1 protein with respect to the adaptation and invasion of different lineages in the vector [8]. E1 T98A substitution had shown increased susceptibility and adaptation to *Ae. albopictus* however E1 211K was not found to be associated with a significant increase in CHIKV infectivity in *Ae.albopictus* and had shown epistatic interaction between position E1-226 and E1- 98 of CHIKV [9].

Besides the specific adaptation pattern exhibited by different lineages to specific vectors ,the strains emerged after 2006 had caused severe pathogenicity such as neurological complications [9,10,11], ocular infections [12], dermatological manifestations [13], hepatitis,severe arthropathy [14] in the infected patients than the strains evolved before 2006.

In this study, we the authors had tried to find the impact of aminoacid substitutions on the structural properties of E1 protein between the two strains of Chikungunya virus isolated before and after 2006 epidemics by identifying the disparity through electrostatic potential and interaction of amino acids in the tertiary structure.

2. MATERIALS AND METHODS

2.1 Viral Isolates

Chikungunya viral isolates [Strain identification No-M 4944 & isolated year- 1963; India] M 60613 & isolated year 2006; India] were obtained from National Institute of Virology, Pune and the isolated virus were reconstituted in Minimum Essential Medium. 100 µl of each strain were inoculated into confluent Vero cell line and Cytopathic effects were observed .Cell line showing CPE was freeze-thawed and centrifuged at 3000 rpm for 15 minutes and the supernatant were stored at -80°C.

2.2 Estimation of Replication Kinetics

Confluent monolayer of cells were infected at multiplicity of infection of 1 and incubated at 37°C for 1 hr. After 1 hr of incubation, 2% maintenance medium was added and incubated at 37 in 5% Co_2. Virus inoculums were then removed at an interval of 24 hrs upto 48 hrs and centrifuged at 3000 rpm for 15 min to remove the supernatant and stored at -80°C for titration. Each experiment was done in triplicates.

Virus titres from cell culture supernatant were quantified. Serial 10 –fold dilutions were made of samples collected at each point and 100 µl of samples of each dilution were added in triplicates in 96 well plates containing confluent monolayer of vero cells. Viral titre was determined as $TCID_{50}$ by Reed and Meunch method.

2.3 Isolation of RNA

Samples were pelleted and to the supernatant 1ml RNAsol was added. The samples were incubated at 70°C for 5 minutes. 200 µl of Chloroform: Isoamyl alcohol [24:1] was added to the mix and centrifuged at 10,000 rpm for 5 minutes at 4°C and to the supernatant added equal volume of Iso-propanol [1:1] and centrifuged at 10000 rpm for 15 minutes at 4°C. Pellet was washed with 500µl of 70% DEPC treated ethanol twice at 9500 rpm for 5minutes.Pellet was dissolved in 40ul DEPC water.

2.4 cDNA Synthesis

1 µg of Total RNA Template and oligo dT primer were mixed with DEPC water to make up the volume to 50 µl of the final reaction mix. Incubated at 65°C for 15min and quick chilled on ice for 5 min. Reverse transcriptase reaction was set up by adding the components in the following order: DTT [0.1M] 5 µl;dNTP Mix [2.5 mM each] 2 µl; RT-Buffer [10X] 5 µl;RNasin [40U/µl] 0.25 µl;MMuLV RT [40U/µl] 2 µl;DEPC water to a final volume of 50.0 µl. The above RT mix was incubated at 42°C for 1hr followed by heat-inactivation of the RT reaction at 94°C for 5 min and snap-Chilled on ice.

2.5 PCR Standardization

The cDNA was then used for PCR amplification of E1 gene, using gene specific primers.

E1-GeneFP-New:
5' – AGAACAGCTAAAGCGGCCACATACC – 3'
E1-GeneRP-New:
5' – CACATGTGCTTCGCTCAATTGCGTG – 3'
E1-Seq-FP1:
5'–GCTGCGGTACAGCAGAGTGCAAGGAC–3'
E1-Seq-FP2:
5' –CAGGCACCATCTGGCTTTAAGTATTGG–3'
E1-GeneRP:
5' – GTTAGTGCCTGCTGAACGACACGCA – 3'.

The reaction mix was prepared by using Template [cDNA] 3.0 ul; Forward Primer 100ng; Reverse primer 100 ng; dNTPs 10 mM;10X Taq Assay Buffer 5.0 µl;FasTaq Enzyme 0.5 µl. PCR I was done using the primers E1-GeneFP-New & E1-GeneRP-New.Expected size of the amplicon was ~522 bp and the PCR amplicon obtained were sequenced using the primer E1-GeneRP-New.

Likewise, PCR II was done using the primers E1-Seq-FP1 & E1-Gene-RP. Expected size of the amplicon was ~1137 bp and the PCR amplicon obtained were sequenced using the primers E1-Seq-FP1; E1-Seq-FP2 & E1-Gene-RP, to read the entire stretch of the gene amplified. PCR was performed for 35 cycles at initial denaturation at 94°C for 5min followed by renaturation at 55°C for 30 sec and annealing at 72°C for 1.30 sec to obtain the ~ 522 bp, and the denaturation at 94°C for 30 sec followed by renaturation at 55°C for 30 sec and annealing at 72°C for 5 min. PCR products obtained were Gel eluted [Figs. 1.1 & 1.2] and purified using Chromous Gel extraction kit.

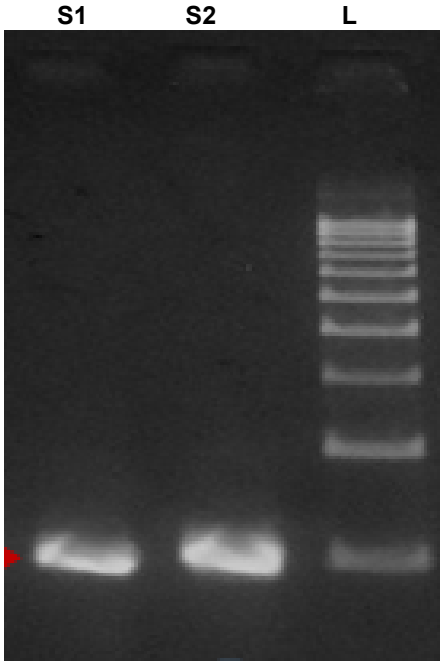


Fig. 1.1. Amplification of E1 gene of Chikungunya virus

Agarose gel electrophoresis demonstrating the 522 base pair of the E1 gene generated by RT-PCR for Chikungunya on 1% agarose gel against 5 Kilo base pair DNA ladder. Lane A shows the strain 1 of ~ 522 amplicon band and Lane B shows the strain 2 of ~ 522 amplicon band. Strain 1 and Strain 2 represents the Asian KC 969207 and ECSA KC 969208 respectively

2.6 Sequencing

The PCR products were sequenced using Big Dye Terminator version 3.1" Cycle sequencing kit in ABI sequencing machine ABI 3500 XL Genetic Analyzer in POP_7 polymer 50 cm Capillary Array following BDTv3-KB-Denovo_v 5.2 protocol.

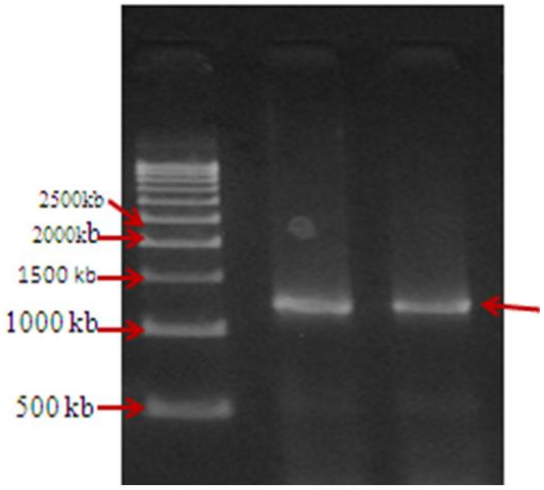


Fig. 1.2. Amplification of E1 gene of Chikungunya virus

Agarose gel electrophoresis demonstrating the 1137 base pair of the E1 gene generated by RT-PCR for Chikungunya on 1% agarose gel against 5kilobase pair DNA ladder. Lane A shows the strain 1 of ~ 1137 amplicon band and Lane B shows the strain 2 of ~ 1137 amplicon band and ladder of 5 kilo basepair. Strain 1 and Strain 2 represents the Asian KC 969207 and ECSA KC 969208 respectively

10 µl sequencing reaction composition was prepared using Big Dye Terminator Ready Reaction Mix: 4 µl; Template [100 ng]: 1 µl; Primer [10 pmol]: 2 µl; Milli Q Water: 3 µl. PCR cycle was performed by 25 cycles with the initial denaturation at 96°C for 1min followed denaturation: 96°C for 10 sec, Hybridization: 50°C for 5 sec and Elongation: 60°C for 4 min. The obtained sequence data were analysed using Seq Scape_ v 5.2. Sequences were assigned the NCBI genbank ID with Asian strain KC969207 and ECSA strain KC 969208.

2.7 Phylogenetic Analysis

Relationships among the aligned amino acid sequences of the Chikungunya virus were determined using Mega version 5.0. The Kimura two-parameter algorithm was applied to calculate the evolutionary distances and the neighbour-joining method was used to construct the phylogram, which was viewed using the treeview program. Bootstrap analysis was performed on 1000 replicas using the programs seqboot and consense to ascertain support for the major branches of the tree. The existing sequences from the NCBI GenBank with the accession numbers were retrieved excluding the sequences collected from mosquitoes and were analysed for the construction of phylogenetic tree to deduce the clade of the subjected query sequence.

Pairwise comparison was performed using Lalign between the protein sequence of the two tested strains.

2.8 Determination of Structural and Functional Variations

The aminoacid sequence of Asian and ECSA of CHIKV were determined and the secondary structure were predicted using Psi-Pred [http://bioinf.cs.ucl.ac.uk/psipred/ psiform.html] [15,16]. Also the coiled coil structures were determined using multicoil [http://groups.csail.mit.edu/cb/multicoil/cgi-bin/multicoil.cgi] by Hidden Markov model [HMM] to predict any significant mutation in the fusogenicity region, if any ,in the coiled coil region [17,18]. The 3D structure and the atomic surface arrangements were identified using the Swiss Model Server. Molecular surface model of proteins and the influence of aminoacid substitution on the structure were calculated with Deep view 3.7 [19,20].

Electrostatic potentials were calculated with partial atomic charges of the Gromos 4.3 A1 force field and with dielectric constants of 4 and 80 for the protein and solvent respectively using Poisson –Boltzmann method. The absolute and relative solvent accessibility of amino acids with substitutions were predicted using NetSurfP [http://www.cbs.dtu.dk/services/NetSurfP/] [21].

3. RESULTS

3.1 Growth Kinetics

Both the strains showed similar characteristic programmed cell death features such as membrane rupture, nuclear condensation, cell fragmentation resulting in total monolayer destruction of Vero cells within 36 hrs of viral inoculation. Although both produced a similar pattern of CPE, the isolates showed a slight difference in their replication kinetics. Strain M 60613 produced 1.5 fold $\log_{10}$ titre increased viral production than the strain M 4944 at the multiplicity of infection 1 after 36 hrs.

3.2 Sequencing Analysis

Phylogenetic analysis revealed the strain M 4944 as Asian clade of Chikungunya virus and shared 99% homology with these strains isolated from India ABN04200, ADG95917and ABN04202.

Strain M 60613 was identified as East central South African strain shared a common ancestor with the S27 African prototype [AAN05102] and with the strains [Ross strain] isolated during 1953 in Tanzania and to the democratic republic of Congo in 1960 as a sub lineage.

Aminoacid substitution between the Asian and African isolates were noted at S72N, T98A, A121S, V142I, S145T, E211K,V215A, S225A, A315V, A322V positions in the extracellular Topological domain of CHIKV [Fig. 2].

3.3 Structure Cum Function Analysis

E1 fusion peptide loop comprises the region from $E_1$73 to $E_1$90 [-VVPFMWGGAYCFCDHENT-] and there were no difference in aminoacid between the Asian and ECSA strains as revealed by sequence analysis. The aminoacid present near the fusion loop showed substitutions at S72N and T98 A positions and thus their electrostatic influence on the fusion loop were analysed.

```
                10        20        30        40        50        60
Seq1    YEHVTVIPNTVGVPYKTLVNRPGYSPMVLEMELLSVTLEPTLSLDYITCEYKTVIPSPYV
        ::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
Seq2    YEHVTVIPNTVGVPYKTLVNRPGYSPMVLEMELLSVTLEPTLSLDYITCEYKTVIPSPYV
                10        20        30        40        50        60

                70        80        90       100       110       120
Seq1    KCCGTAECKDKSLPDYSCKVFTGVYPFMWGGAYCFCDTENTQLSEAHVEKSESCKTEFAS
        ::::::::::.:::::::::::::::::::::::::.:::::::::::::::::::::::
Seq2    KCCGTAECKDKNLPDYSCKVFTGVYPFMWGGAYCFCDAENTQLSEAHVEKSESCKTEFAS
                70        80        90       100       110       120

               130       140       150       160       170       180
Seq1    AYRAHTASASAKLRVLYQGNNVTVSAYANGDHAVTVKDAKFIVGPMSSAWTPFDNKIVVY
        .::::::::::::::::::::.::.:::::::::::::::::::::::::::::::::::
Seq2    SYRAHTASASAKLRVLYQGNNITVTAYANGDHAVTVKDAKFIVGPMSSAWTPFDNKIVVY
               130       140       150       160       170       180

               190       200       210       220       230       240
Seq1    KGDVYNMDYPPFGAGRPGQFGDIQSRTPESEDVYVNTQLVLQRPSAGTVHVPYSQAPSGF
        ::::::::::::::::::::::::::::::.:::.::::::::::.:::::::::::::::
Seq2    KGDVYNMDYPPFGAGRPGQFGDIQSRTPESKDVYANTQLVLQRPAAGTVHVPYSQAPSGF
               190       200       210       220       230       240

               250       260       270       280       290       300
Seq1    KYWLKERGASLQHTAPFGCQIATNPVRAMNCAVGNMPISIDIPDAAFTRVVDAPSLTDMS
        ::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
Seq2    KYWLKERGASLQHTAPFGCQIATNPVRAMNCAVGNMPISIDIPDAAFTRVVDAPSLTDMS
               250       260       270       280       290       300

               310       320       330       340       350       360
Seq1    CEVPACTHSSDFGGAAIIKYAASKKGKCAVHSMTNAVTIREAEIEVEGNSQLQISFSTAL
        ::::::::::::::.:::::.::::::::::::::::::::::::::::::::::::::::
Seq2    CEVPACTHSSDFGGVAIIKYAVSKKGKCAVHSMTNAVTIREAEIEVEGNSQLQISFSTAL
               310       320       330       340       350       360

               370       380       390       400       410       420
Seq1    ASAEFRVQVCSTQVHCAAECHPPKDHIVNYPASHTTLGVQDISATAMSWVQKITGGVGLV
        ::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
Seq2    ASAEFRVQVCSTQVHCAAECHPPKDHIVNYPASHTTLGVQDISATAMSWVQKITGGVGLV
               370       380       390       400       410       420

               430
Seq1    VAVAALILIVVLCVSFSRH
        ::::::::::::::::::::
Seq2    VAVAALILIVVLCVSFSRH
               430
```

Fig. 2. Comparative analysis performed between Asian and East Central South African strains by LAlign

Calculations of the Electrostatic potential were performed with Swiss-PDB viewer. The orientation of structures were identical in all panels and the electrostatic calculations were performed in an identical manner. The electrostatic potential calculated was identical /same irrespective of the amino acid substitution observed between Asian and ECSA strains at positions 72, 98, 121, 142, 145, 215, 225, 315 and 322 positions and had not produced any significant change in electrostatic potential except at the position 211 [Figs. 3.1 to 3.6]. The differences in the net solvent accesability of the exposed and buried aminoacids between the Asian and ECSA strain are shown in Table 1.

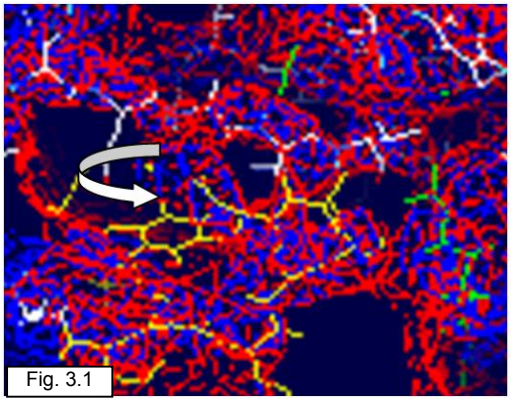


Fig. 3.1

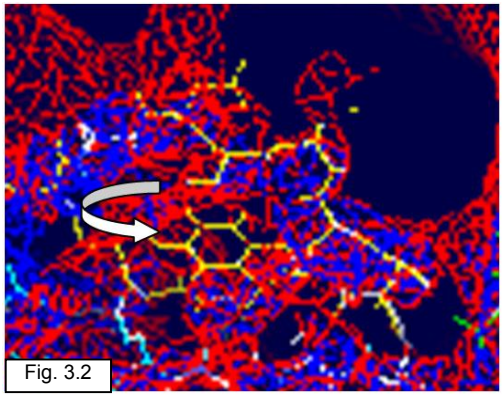


Fig. 3.2

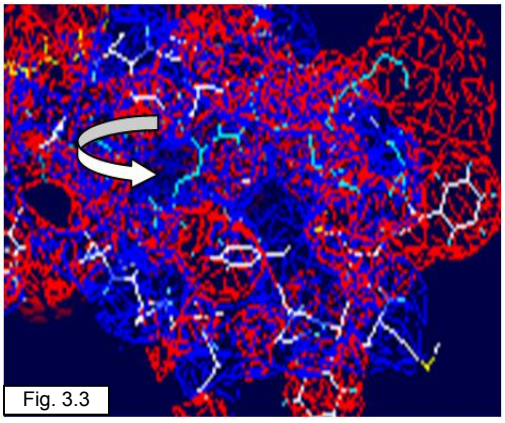


Fig. 3.3

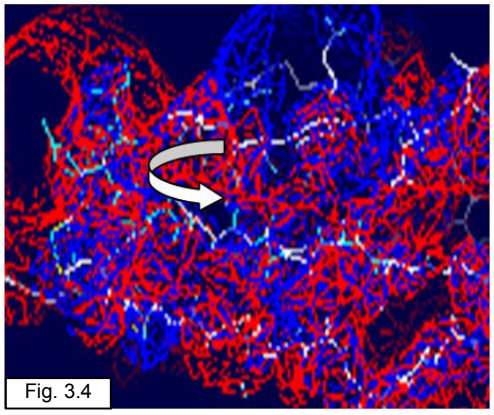


Fig. 3.4

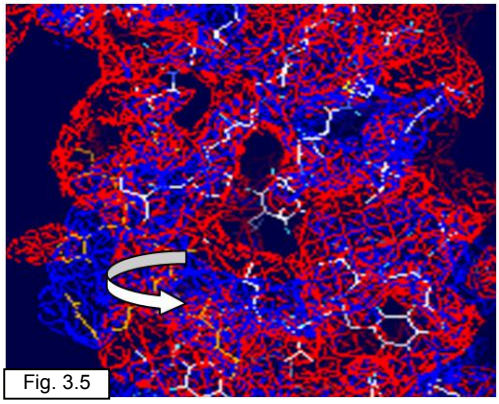


Fig. 3.5

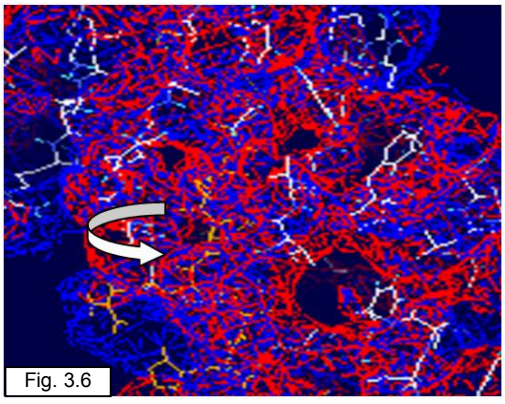
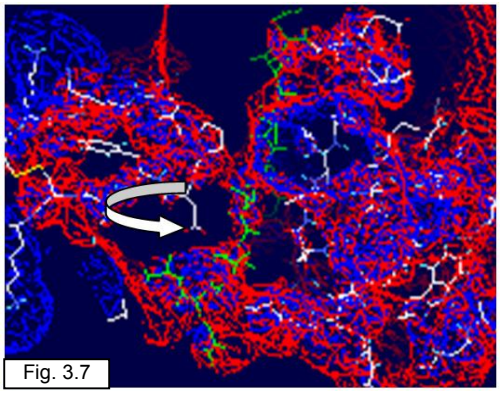


Fig. 3.6



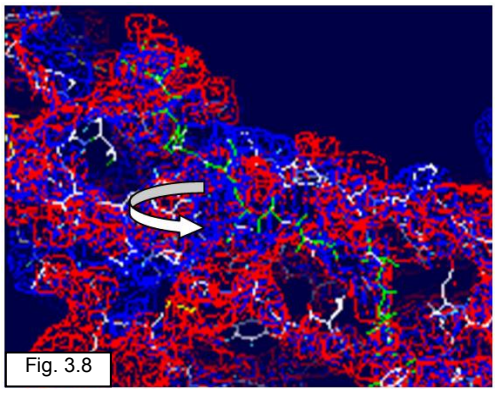


Fig. 3. Electrostatic potential of the amino acids on the homology modelled structure of the East Central South African strain of Chikungunya virus

Represents the electrostatic potential exhibited by specific aminoacids on the surface structure of Chikungunya virus using slab view under Swiss PDB viewer. Yellow colour indicates the location of S72 and N 72 residues in Asian [Fig. 3.1] and ECSA [Fig. 3.2]; Blue colour indicates the location of T98 and N 98 residues in Asian [Fig. 3.3] and ECSA [Fig. 3.4]; Orange colour indicates the location of A 121 and S 121 residues in Asian [Fig. 3.5] and ECSA [Fig. 3.6].Green colour indicates the location of E211 and K211 residues in Asian [Fig. 3.7] and ECSA [Fig. 3.8] respectively. The aminoacid substitution at position 72, 98 and 121 had not produced any influence /difference on the electrostatic potential between the Asian and ECSA. Mutation at 211 had caused a shift in the electrostatic surface properties ie more negative charge [red] due to the aminoacid glutamate on the Asian strain [Fig. 3.7] and more positive charge [blue] due to the aminoacid lysine on the ECSA strain [Fig. 3.8]

Table 1. Relative and absolute solvent accessibility of the substituted amino acids between Asian and ECSA Chikungunya virus

Position	Amino acid		Nature of the aminoacid		Relative and absolute solvent accessibility		Z fit score	
	Asian	ECSA	Asian	ECSA	Asian	ECSA	Asian	ECSA
72	S	N	Exposed	Exposed	0.682 79.966	0.671 98.278	-0.543	-0.729
98	A	T	Exposed	Exposed	0.323 35.595	0.336 46.589	-0.729	-0.648
121	A	S	Buried	Buried	0.120 13.224	0.127 14.920	-0.554	-0.681
142	V	I	Exposed	Exposed	0.324 59.848	0.349 53.580	-0.078	-0.099
145	S	T	Exposed	Exposed	0.327 38.289	0.312 43.274	-0.666	0.754
211	E	K	Exposed	Exposed	0.593 116.982	0.569 103.597	-0.280	-0.160
215	V	A	Buried	Buried	0.167 18.943	0.172 25.606	-0.980	-0.880
225	S	A	Exposed	Exposed	0.623 72.992	0.623 68.677	-0.962	-0.959
315	A	V	Buried	Buried	0.160 17.676	0.171 26.252	-0.547	-0.483
322	A	V	Buried	Buried	0.256 40.823	0.266 28.222	-1.382	-1.100

3.4 Effects of A to S Mutation and E to K Mutation in Structure Formation of Asian and ECSA Strain

Further analysis on the conformational changes of the specific mutations had shown that all the exposed and buried aminoacids in E1 protein of both Asian and ECSA were the same except at A to S in 121 position. In the Asian strain, O terminal and N terminal of Alanine 121 had formed hydrogen bond with the N terminal Aspargine 45[2.99 A°] and with the oxy terminal

of Tyrosine 46 [2.85 A°]. In contrast the mutation [shift from A to S] at 121 position of ECSA strain had resulted in the local rearrangement showing interaction between OG terminal of Serine 121 forming Hydrogen bond with oxygen terminal of Tyr 46 [2.72 A apart] and also another bond formation with N terminal of Tyr 46[2.95 A apart] [Fig. 4. 2]. Oxygen Terminal of serine 121 had formed Hydrogen bond with Asp 45 [3.00 A apart] and N terminal of Ser 121 with Oxy terminal of Tyr 46[2.85 A]. Consequently in the ECSA, the OG terminal of 121S is found to form two new additional intermolecular bonds with the same monomer at its backbone. Thus this movement had resulted in the extension of two intermolecular H-bonds between these residues in ECSA and loss of two intermolecular hydrogen bonds in Asian lineage [Fig. 4.1].

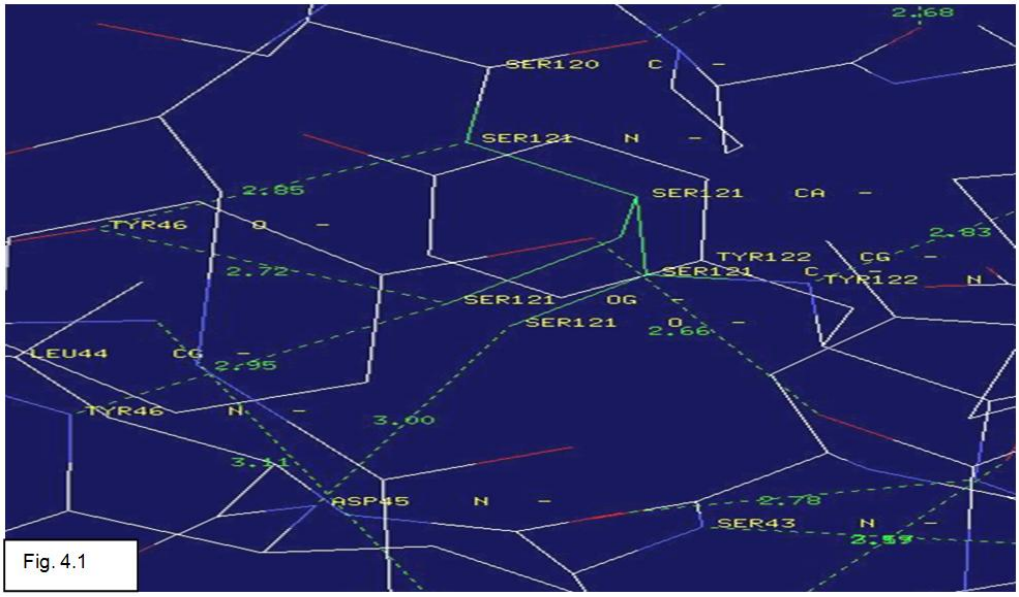


Fig. 4.1

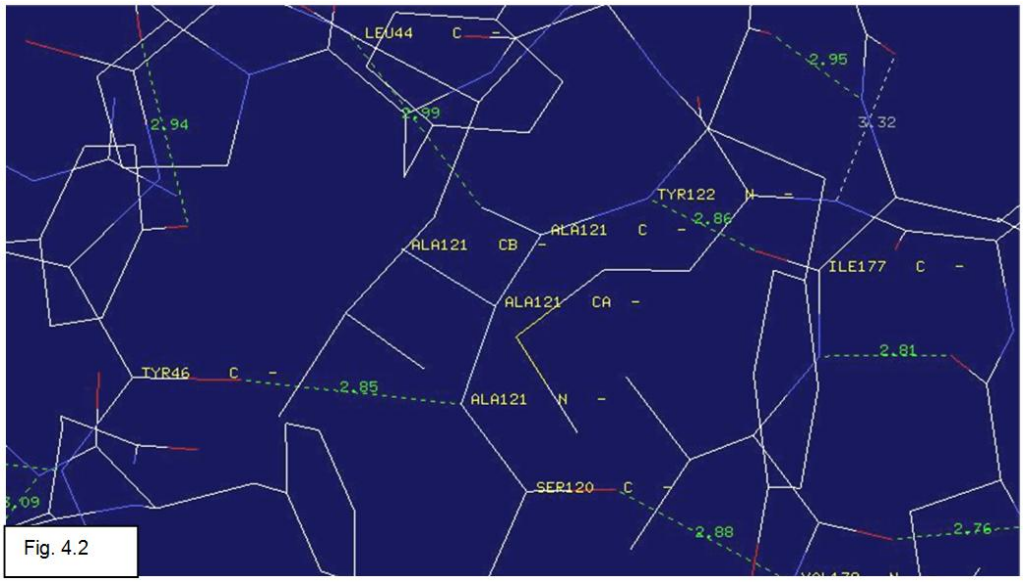


Fig. 4.2

Fig. 4. Differences in the aminoacid interaction due to the change in A ⟶ S at 121 position in the E1 protein of Chikungunya virus

Displaying the amino acid interaction of Serine 121 [ECSA] extending four hydrogen bonds with the adjacent monomers Tyrosine 46 and Asparagine 45 [Fig. 4.1] and Alanine 121 [Asian] extending two hydrogen bonds with the adjacent monomers Tyrosine 46 and Asparagine 45 [Fig. 4.2]

4. DISCUSSION

E1 protein plays a major role in the fusion of virus to the host cell receptors and also in vector Specificity hence we studied the impact of aminoacid substitutions of E1 protein on the structural properties between the two different strains of Chikungunya virus. Serial passaging of virus in vero cells were carried out till 10^{th} passage and the sequencing analysis both at the intermediate and 10^{th} passage had not revealed any induced nonsynonymous mutation/de nova mutation in E1 protein of both the Asian and ECSA strain signifying the possible role of host immunity as an important selective pressure for the establishment, dominance and emergence of strains during epidemics.

A slight Variation in the secondary structure between the Asian and ECSA were observed. The number of random coils observed were the same in both the strains however variations in beta turn ,alpha helix and extended beta strand were detected as analysed by SOPMA. But the superimposition of the two different structures did not reveal any differences between them.

Both the strains used in our study were wild type E226A and had not the A226V mutation, a factor involved in the Cholesterol dependence of vectors but had A226G mutation signifying that the two isolates were virulent. Earlier studies on Chikungunya viruses had shown that S, T,G,P aminoacids in 226 position in the E1 protein were almost indistinguishable from parental wild type viruses in their virulence properties but the strains showing I,F,M or H and L had shown slight attenuation or complete attenuation respectively [22]. The most conserved aminoacids G91 and H230 were present in the two isolates that are found to be playing a prime role in fusogenicity [23].

We hypothesised that an increased 1.5 fold magnitude growth of ECSA than Asian strain in Vero cells could be due to mutations observed in the membrane fusion protein and thus tried to explore the influence of amino acid substitution on the structure and functional characteristics of E1 protein by Molecular Modelling.

However E211K amino acid substitution [E to K] showed marked difference in the expression of electrostatic potential on the neighbouring amino acids. The strain with K substitution [ECSA strain] increased the overall electrostatic potential of the neighbouring amino acids on the topological domain of the virus. K and E residues were positively and negatively charged aminoacids respectively, their opposite charges had slightly affected the isoelectric point [pI] of the protein with a significant difference of Asian 6.51 from ECSA 6.79 [Figs. 3.7 & 3.8]. Thus the difference in electrostatic potential shown by the two different clades could affect the interaction with the cell receptors during fusogenicity in the Topological domain I of CHIKV as the aminoacid H230 [conserved aminoacid involved in the fusogenicity] is present near the E211 region. The change in aminoacid from Lysine [Asian] to glutmate [K] in ECSA had shown a higher positive potential in ECSA.

In order to further understand the difference in the monomer interaction, the substituted aminoacids were analysed by computing Hydrogen bond formation and the distances with other monomers were analysed using Swiss PDB viewer v.4.01. The exposed aminoacids that had undergone substitution did not show any loss in the intermolecular bond formation with the adjacent monomers and were stable, however the buried aminoacid substitution A to S had shown significant difference in the intermolecular hydrogen bond formation with the monomer present at its backbone.

During fusion, E1 inserts into the target membrane via the fusion loop forms a core trimer composed of domains I & II and refolds to a hairpin like confirmation in which DIII and the stem [DIII] pack against the central core trimer. This refolding reaction moves the TM domain and the fusion loop to the same side of the trimer, bringing the viral and target membranes together and driving membrane fusion [11,18]. Thus the low PI of Serine [5.8] in comparison to alanine [6.02] at 121 position of ECSA along with the slight higher electrostatic potential exhibited by lysine at 211 position on the surface structure of neighbouring amino acids could better influence the fusogenicity and the formation of stable trimer in ECSA than the Asian strain .The study also highlights that the mutation were higher and notable only in the extracellular topological domain 1 of E1 protein of CHIKV and the other domains [II and III] ,fusion loop were highly conserved. Thus the aminoacid substitutions observed especially at 121 and 211 positions could highly influence the fusogenicity and interaction with the host receptors.

E1 protein plays major role in pathogenicity by remarkable cell tropism and mediating entry into

susceptible cells. The aminoacid substitution in Japanese encephalitis virus at 138[Glutamic acid to lysine] and 123 [methionine to lysine] [24,25] and 335 [Glutamic acid to Lysine]in the HN glycoprotein of mumps virus had highly attributed towards neurotropism [26] likewise the specific mutations at E211K position from glutamic acid to lysine in ECSA strain could also feature for higher virulence as the strains emerged after 2006 epidemics were, in particular, found to exhibit neurological complications. Thus further molecular insights and *in vivo* experiments are also warranted to support our findings.

5. CONCLUSION

As the significant conformational change occur at the aminoacid positions 121 and 211 lies near the fusion loop, the difference in aminoacid position between the two strains could influence their fusogenicity and stable trimer formation. This impact had provided a new insight into the influence of the aminoacid substitutions pertaining to the three dimensional structural of E1 protein between the strains of Chikungunya virus.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Santiago F. Elena, Rafael Sanjuán. Adaptive value of high mutation rates of RNA viruses: Separating causes from consequences. J Virol. 2005;79(18): 11555–11558.
2. Sara M. Volk, Rubing Chen, Konstantin A. Tsetsarkin, Paige Adams A, Tzintzuni I. Garcia, et al. Genome-scale phylogenetic analyses of Chikungunya virus reveal independent emergences of recent epidemics and various evolutionary rates. J Virol. 2010;84(13):6497-6504.
3. Ann M. Powers, Aaron C. Brault, Robert B. Tesh, Scott C. Weaver. Re-emergence of Chikungunya and o'nyong-nyong viruses: Evidence for distinct geographical lineages and distant evolutionary relationships. Journal of General Virology. 2000;81(2): 471-479.
4. Schuffenecker I, Iteman A, Michault S, Murri, Frangeul L, Vaney MC, et al. Genome microevolution of Chikungunya viruses causing the Indian Ocean outbreak. PLoS Med. 2006;3:e263.
5. Chatterjee PK, Eng CH, Kielian M. Novel mutations that control the sphingolipid and cholesterol dependence of the Semliki Forest virus fusion protein. J Virol. 2002; 76:12712-12722.
6. Li L, Jose J, Xiang Y, Kuhn RJ, Rossmann MG. Structural changes of envelope proteins during alphavirus fusion. Nature. 2010;468:705-708.
7. Tsetsarkin KA, Vanlandingham DL, McGee CE, Higgs S. A single mutation in Chikungunya virus affects vector specificity and epidemic potential. PLoS Pathog. 2007;3:e201.
8. Tsetsarkin KA, CE McGee, VolK SM, Vanlandingham DL, Weaver SC, Higgs S. Epistatic roles of E2 glycoprotein mutations in adaption of Chikungunya virus to *Aedes albopictus* and *Ae. aegypti* mosquitoes. PLoS One. 2009;4:e6835.
9. Ganesan K, Diwan A, Shankar SK, Desai SB, Sainani GS, Katrak SM. Chikungunya encephalomyeloradiculitis: Report of 2 cases with neuroimaging and 1 case with autopsy findings. American Journal of Neuroradiology. 2008;29:1636-1637.
10. Lewthwaite P, Vasanthapuram R, Osborne JC, Begum A, Plank JLM, Veera SM, et al. Chikungunya virus and central nervous system infections in children, India. Emerg Infect Dis. 2009;15(2):329–331.
11. Sanchez-San Martin C, Liu CY, Kielian M. Dealing with low pH: Entry and exit of *Alphaviruses* and *Flaviviruses*. Trends Microbiol. 2009;31:56-60.
12. Rose N, Anoop TM, John AP, Jabbar PK, George KC. Acute optic neuritis following infection with Chikungunya virus in Southern Rural India. Int J Infect Dis. 2010;15(2):e147-e150.
13. Bandyopadhyay D, Ghosh SK. Mucocutaneous features of Chikungunya fever: A study from an outbreak in West Bengal, India. Int J Dermatol. 2010;47: 1148-1152.
14. Ozden S, Huerre M, Riviere JP, Coffey LL, Afonso PV, Mouly V, et al. Human muscle satellite cells as targets of Chikungunya virus infection. PLoS ONE. 2007;2:e527.
15. Cuff JA, Clamp ME, Siddiqui AS. JPred: A consensus secondary structure prediction server. Bioinformatics. 1998;14:892-3.
16. Deleage G, Combet C, Blanchet C, Geourjon C. Antheprot: An integrated protein sequence analysis software with client/server capabilities. Comput Biol Med. 2001;31:259-67.

17. McGuffin LJ, Bryson K, Jones DT. The PSIPRED protein structure prediction server. Bioinformatics. 2000;16:404-5.
18. Delorenzi M, Speed T. An HMM model for coiled coil domains and a comparison with PSSM-based predictions. Bioinformatics. 2002;18(4):617-625.
19. Guex N, Peitsch MC. SWISS-MODEL and the Swiss-PdbViewer: An environment for comparative protein modeling. Electrophoresis. 1997;18:14-23.
20. Kopp J, Schwede T. The SWISS-MODEL repository of annotated three-dimensional protein structure homology models. Nucleic Acids Res. 2004;32:230-4.
21. Rost B, Yachdav G, Liu J. The predict protein server. Nucleic Acids Res. 2004; 32:W321-W326.
22. Tsetsarkin KA, Chen R, Leal G, Forrester N, Higgs S, Huang H, et al. Chikungunya virus emergence is constrained in Asia by lineage-specific adaptive landscapes. Proc Natl Acad Sci USA. 2011;108:7872-7877.
23. Szu-Cheng Kuo, Ying-Ju Chen, Yu-Ming Wang, Pei-Yi Tsui, Ming-Der Kuo, Tzong-Yuan Wu, et al. Cell-based analysis of Chikungunya virus E1 protein in membrane fusion. Journal of Biomedical Science. 2012;19:44.
24. Monath TP, Arroyo J, Levenbook I, Zhang ZX, Catalan J, Draper K, et al. Single mutation in the flavivirus envelope protein hinge region increases neurovirulence for mice and monkeys but decreases viscerotropism for monkeys: Relevance to development and safety testing of live, attenuated vaccines. J Virol. 2002;76: 1932-1943.
25. Sumiyoshi H, Tignor GH, Shope RE. Characterization of a highly attenuated Japanese encephalitis virus generated from molecularly cloned cDNA. J Infect Dis. 1995;76:1144–1151.
26. Gerardo Santos-López, Thomas Scior, María del Tránsito Borraz-Argüello, Verónica Vallejo-Ruiz, Irma Herrera-Camacho, José Tapia-Ramírez4and Julio Reyes-Leyva. Structure-function analysis of two variants of mumps virus hemagglutinin-neuraminidase protein. The Brazilian Journal of Infectious Diseases. 2009;13(1):24-34.

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