Chikungunya Virus Vaccine Development: Through Computational Proteome Exploration for Finding of HLA and cTAP Binding Novel Epitopes as Vaccine Candidates

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Abstract
Chikungunya virus is a major arbovirus of great public health concern in the whole world, but no vaccine is yet available, still advance therapeutic treatment and effective vaccines are in progress. The present multistep screening and structural binding analysis with CHIKV proteome exploration can be crucial in the development phase of CHIKV epitope based vaccine. The approach employed in two phases (i) Sequence based screening of peptides through propred and IEDB Server (ii) Structure based study through autodocking and NAMD VMD simulation analysis. Among all 29 extracted peptides, only two peptides 2LLANTTFPC10 of protein E3 and 98VNSVAIPLL106 of protein nsP3 were observed most prominent over all consider parameters such as peptide conserve nature, supertype population coverage, TAP binding, docking and simulation study. During docking interaction study, the best peptide and allele docked complexes such as 2LLANTTFPC10–B*0702 allele and 98VNSVAIPLL106–A*0301 allele exhibited best binding energy of − 3.13 kcal/mol and − 3.19 kcal/mol, respectively, with stable bonding patterns and their motion during NAMD simulation which confirm conserve peptide and allele stable interaction. The current study also exhibited the good docking interaction of both peptides 2LLANTTFPC10 and 98VNSVAIPLL106 with c TAP1 protein (1jj7 -PDB ID) cavity which confirm as a channel passageway to peptide transport through the cytoplasm to lumen of ER during antigen processing and presentation. Overall, this multistep screening and crosscheck structural binding analysis with an exploration of the complete proteome of CHIKV can be a novel step in the development of CHIKV epitope based vaccine as well as diagnostic development with aspect of time, cost and side effects.

Keywords Chikungunya · Epitope · Peptide · Simulation · TAP, Vaccine

Introduction
Chikungunya virus is a major arbovirus of great public health concern in the whole world which belongs to toga-viridae family (Sanyaolu et al. 2016). As arbovirus, CHIKV transmission in humans occurs through mosquito vectors Aedes aegypti and Aedes albopictus. CHIKV infection cause generally chikungunya fever but its infection severity result morbidity with chronic stages such as poly-arthritis and prolong myalgia (Cunha et al. 2020). In the acute-infected individuals other severe complication like gastrointestinal upset, encephalitis, depression, lung, kidney, heart dysfunction, loss of vision, sleep disorder and memory loss being perceived (Sanyaolu et al. 2016; Qamar et al. 2018). The risk of Chikungunya may increase in the older patient more than 65 years and younger ones, posses more severe symptoms like neurological, cardiac and mortality also (Horwood and Buchy 2015). The present time, WHO also recorded high rate of CHIKV infections in India as well as rest of the developing countries due to urbanization activities, poor sanitization and weak mosquito vector control. Along with this, same vector and similar infection of CHIKV and Dengue virus which misleads treatment plans of both infections, causing major problems for health workers (Dutta et al. 2017; Saxena and Mishra 2021). On other side, the available
therapeutic drugs and conventional vaccines lack the potential to address this global problem of CHIKV infection (WHO 2020; Weaver et al 2012) still advance therapeutic treatment and effective vaccines are in progress. CHIKV is a single positive-strand RNA virus with genome size 11.8 kb which translated in to structural capsid protein, envelope protein (E1, E2, 6 K and E3) and enzymatic non structural nsP1, nsP2, nsP3 and nsP4 proteins (Lee & Chu 2015; Qamar et al. 2018).

In the development of vaccine research, a specific approved or therapeutic vaccine for CHIKV is not yet available (WHO 2020). Therefore, the only option to verify the extent of CHIKV is quarantine and vector control with symptomatic therapeutic treatment at this time. In some way, this is effective only in the developed countries because of well develop techniques, but in the developing countries, these methods not working properly because of the large population and lacking of resources (Silva and Dermody 2017). So that's why persistency of this disease has remained very high because of insufficient therapeutic and control of this disease. Consequently, all facts demands specific therapeutic treatment as well as effective and advance vaccines to control the severe CHIKV infections globally. The traditional vaccines, likes- heat-killed and live attenuated vaccines having some safety issues because of whole microorganism administration as well as time and cost are also major issues. (De Groot et al. 2002; Sharma et al. 2018). In current scenario, the advent of immunoinformatics tools and databases are helping in the development of multi epitope vaccines, which rising as a new hope to resolve all above limitations of conventional vaccines for human pathogenic viruses, bacteria (Joshi and Kaushik 2020), fungi (Akhtar et al. 2021) as well as veterinary pathogens also (Jain et al. 2021).

Vaccines based on epitopes are better than conventional vaccines and can also overcome side effects and safety issues (Krishnan et al. 2020; Srivastava et al. 2020). In the development of epitope based vaccines requires complete knowledge of immunology to initiate immune response. The epitope peptides cannot generate immunity alone; epitope should attach to Major Histocompatibility Complex (MHC) membrane proteins of host cell. Generally MHC molecules in human are called Human Leukocyte Antigen (HLA) which show high diversification in human population which raises the issue of population coverage during development of vaccines (Gupta and Kumar 2020a,b). To initiate an immune response towards an antigen; it has to undergo antigen processing and presentation process under which range of epitopes were generated to bind with HLA membrane molecules. Different ethnic population shows variations in binding pattern between HLA alleles and epitope. Therefore, each ethnic populations can be represent by a group of HLA alleles called as Supertypes and those epitopes bind with these supertype alleles group known as superantigens. The idea of supertypes which is representing a particular population has lower the chance of antigen escape from immune response as well as population coverage issues. (Kangueane et al. 2005; Shekhar et al.2012).

During development of epitope based vaccine, screening of highly conserved epitopes along with different supertypes alleles binding analysis strengthened the finding of best superantigen as vaccine reagents which were already shown in several other viruses vaccine development such as H1N1, JEV, DENGUE and WNV etc. ( Kumar et al. 2013; Sharma et al. 2014, 2021). Recently, other than these human pathogens zika virus, fungal pathogen as well as veterinary pathogens also showed potential results in development of epitope vaccine (Sharma et al. 2019; Jain et al. 2021). In several studies, epitope based vaccine immunizations against various infections showed significant results with proven cellular as well as humoral immunity (Hajissa et al. 2019). Approaches using concept of HLA supertype, with conserve epitope nature have been proven boon for mankind against various infectious disease which change the trends in various vaccine development approaches (Saxena and Mishra 2020). In short, several significances of epitope based vaccines such as complete proteome representation through conserve epitopes, population coverage, less experimental burden, time, cost and doses overcome various issues with conventional vaccines (Krishnan et al. 2021).

Along with this, the process of antigen processing and presentation involved number of proteins such as Transporter Associated Protein (TAP) which embedded in rough endoplasmic reticulum (RER) membrane. The TAP worked as carrier for cytosolic epitopes to transport them through cytosol towards HLA molecule for presentation over membrane. These presented epitopes with HLA alleles make complex with T cell receptors to activate T cell to give immune response. Therefore, cytosolic molecular pathways showed binding of epitopes with TAP and HLA molecules as a major factor to generate immunogenicity against antigen peptide (Procko and Gaudet 2009; Gaudet and Wiley 2001). TAP binding analysis of top screened conserved and superantigenic peptides strengthen the vaccine candidacy of an epitope with more positivity (Sharma et al.2017; Mishra et al. 2020).

The present work is targeted to find most potential vaccine candidates from whole CHIKV proteome with least time as well as least experimental load which is ultimately reducing the burden of CHIKV infection globally. Therefore, this study started with the finding of the range of epitopes from each protein of virus which will be conserved and superantigenic through a sequence based epitope–alleles binder algorithms. After that, these conserved peptides and HLA alleles were generated in their structural models by PEPstrMODE and Swiss-prot for further structural binding
analysis by docking. The binding of conserve superantigen epitopes with HLA molecule as well as cTAP substantiate that the epitopes generated during antigen processing were well transported with cTAP through cytosol and presented with HLA molecules over the membrane. The superantigenic epitopes peptides which showed best binding with HLA molecules were further docked with cTAP to confirm whether these peptides are really processed and presented during cytosolic pathway. Finally, the most potential candidates were undergone for molecular simulation study to confirm stable binding between Epitope and HLA molecule by NAMD-VMD. Overall, this multistep screening and crosscheck structural binding analysis with an exploration of the complete proteome of CHIKV can be a novel step in the development of CHIKV epitope based vaccine.

**Methodology**

In the present study, NCBI database (http://www.ncbi.nlm.nih.gov/entrez) was used to access fasta sequences of all required proteins of Indian CHIKV study strain Accession No. EF210157.2 (Santhosh et al. 2009) as well as all geographical CHIKV regions strains such as India, Italy, Senegal, Thailand and USA. After retrivation of all required sequences of proteins, this study will be divided in two phases such as sequences based study and structure based study. Sequences based study involve the screening of epitopes from study strain, conservancy analysis, supertype analysis pI analysis and finally cross check with other algorithms to validate them. The structure based study was done to confirm the potential of epitopes to bind with HLA molecule by docking and simulation study, to generate an immune response. Prepared and Propred I was employed to retrieve HLA class II and Class I binding epitopes with recommended parameters. Therefore, the epitopes comparative analysis with all geographical regions strains were done to observe variations and conserve nature of propred screened epitopes with the help of IEDB platform. Along with, mutated or varied all epitopes were tested for their pI value. The above all screened epitopes through propred platform were further tested by using IEDB Artificial Neural Network (ANN) and (SMM) based algorithms to cross check the results.

**Extraction of T Cell Binding Epitopes by Sequence Based Study**

The sequence based study will extract out large pools of epitopes through Propred and Propred I server. Propred server was working out for finding of HLA class II binding peptide epitopes for 51 HLA alleles (Singh and Raghava 2001) as well as Propred I was employed for HLA class I binding peptide epitopes for 47 HLA alleles with recommended parameters (Singh and Raghava 2003). MHC Pred 2.0 were used to for epitope TAP binding analysis (Guan et al. 2006). Above extracted epitopes under gone for validation by various Algorithms such as SMM and ANN (Nielsen et al. 2003) by IEDB server.

**Epitope Conserve Nature Analysis, Among Different Geographical Regions**

Extracted CHIKV T cell epitopes were employed to epitope conserve nature analysis, among different geographical regions such as India, Italy, Senegal, Thailand and USA. IEDB epitope conservancy study tool has been used to perform this analysis and required protein sequences of all proteins of different geographical strains were extracted from NCBI database. IEDB conservancy analysis was done by applying minimum five sequences of the same protein randomly for all geographical regions (Bui et al. 2007). Epitopes conservancy was done for all above five different geographical regions to get entirely conserved T cell epitopes. Altogether epitopes were kept in in three groups (i) entirely conserved (ii) having only a single mutation with 80% conservancy (iii) more than one mutation with less than 80% conservancy. Among these three groups, the epitopes having more than one mutation with less than 80% conservancy were taken out from further study and rest epitopes means single mutated and entire conserve epitopes were taken in consideration for further cross check and rigid screening procedure.

**Finding of Common T Cell and B Cell Epitopic Regions in the Whole CHIKV Proteome**

BC pred method algorithm was used to find linear B cell epitopes regions of 20 amino acid from whole CHIKV proteome (El-Manzalawy et al. 2008; Terry et al. 2015). B cell and T cell epitopes interaction with antibody and MHC molecule, respectively, is must for neutralizing the antigen. This finding will reveal the common peptide region, which includes both T cell and B cell epitopes region and have possibility to initiate both B cell and T cell immune response (Saxena and Mishra 2020).

**HLA Supertype Study for CHIKV T Cell Epitopes to Cover Maximum Population by Vaccine Candidates**

The concept of HLA supertype in addition to above epitope conservancy analysis will increase more potency to cover maximum population by sorted conserve epitopes. As already known there are five (A3, A2, A24, B15, B7) HLA class I supertypes (23 HLA alleles) and five (Main DP2,
DP2, DR3, DR2, DR) HLA class II supertypes (14 HLA alleles) which cover the maximum population (Reche and Reinherz 2005). The extracted promising conserve epitopes will studied for binding analysis to all HLA alleles supertype groups in comparison with already known superantigen positive controls to validate epitopes super-antigenicity. The known +ve controls which have been taken in the study are 141STLPETTVV149 peptide of Hepatitis core protein (Accession No.CAA59535) and 260ILRGSAVH573 peptide of H1N1 Nucleoprotein (P03466) (Sharma et al. 2021; Ansari et al. 2009). This study was done by using IEDB platform.

Structural Binding Study of Extracted T Cell Epitopes to Relevant HLA Supertype Alleles and cTAP Protein

Before going to binding study between T cell epitopes and relevant alleles, the three dimensional structural models of each peptide and Supertype alleles were generated through PEPstrMOD and Swiss prot, respectively (Singh et al. 2015; Saxena and Mishra 2020) which have been required to perform all docking and simulation study. The present docking study was done by Autodock 4.2 (Morris et al. 2009). In all docking experiments all recommended parameters were applied to extract the file.pdbqt. The autodock results were observed and analyzed with autodock tools to confirm epitope and alleles binding.

MD Simulation Analysis of Top Docked HLA Allele and Epitopes Binary Complexes

The molecular dynamic simulation (MD) of allele and epitope complexes and their physical interactions within in time window during docking were performed by NAnoscale Molecular Dynamics (NAMD) (James et al. 2005) and their visual assessment done by Visual MD platform (VMD) (Humphrey et al. 1996). For simulation analysis the three dimensional models of each epitopes and alleles were optimized by employing recommended force field parameters such as potential energy of bonding and nonbonding interactions with possible variations. To run NAMD the PSF building tool was used to generate.PSF structure file through.PDB file by extracting all atomic coordinates, velocity, force and sequence data. The pdb files are most necessary raw data for NAMD due to its high accuracy. The DCD trajectory file was created by NAMD and Visual MD (VMD) generated RMSD.TCI source file. During the whole simulation process the RMSD values were stored in a RMSD.DAT file with regard to time frame and RMSD graph was obtained. This molecular simulation of protein–protein interaction will show physical stable bonding patterns and their motion during interaction which confirm epitope conserve peptide stable interaction with HLA alleles (Mishra et al. 2020; Peele et al. 2020).

Results

The present hierarchical approach will result finest T cell peptide epitopes as most promising candidates for vaccine as well as diagnosis of CHIKV infection.

Finding of Promising T Cell Peptide Epitopes of CHIKV Through Sequence Based Analysis

The complete proteome of all 8 proteins undergone in predp analysis to give a range of epitopes with sequence based matrix algorithm by keeping 5% threshold value. These large numbers of epitopes further sorted on the basis of HLA allele frequency of allele binding, conservancy, Tap binding nature. Among all, top most 29 identified epitopes, six structural protein epitopes viz. 2LLANTTFPC10, 31KGRVVAIVL9, 33LKIQVSLQI41, 490AALILIVVL498, 274LILIVVLC279, 418LQISVFSTAL426 and three nonstructural protein epitope 98VNSVAIPLL106, 93VTRLGVNSV103, 187VLLPNVHTL195 were exhibited more than 90% conservancy with high coverage of population through supertype analysis (Table 1). Furthermore, the peptide 39IDNADLAKL47 of capsid protein also showed good binding with HLA class I alleles with completely conservancy.

In the Table 1, under Different Geographical region of CHIKV section, the amino acid letter with green color in peptide shows amino acid variation or mutation in the other Geographical region.

Explanation with example for CHIKV Geographical region section: YEKEPEE(S3,K4,K5)TL, here original peptide is YEKEPEETL. Glutamic acid (E) seventh residue is mutated with Serine (S) in 3rd (Senegal) geographical region, Lysine (K) in 4th (Thailand) and 5th (USA) geographical region.

Conserve Nature of Extracted Peptide Epitopes

During IEDB analysis of conserve nature of extracted peptide epitopes, 8 HLA class II binding peptides and 17 HLA class I binding peptides were found conserve completely. The extracted HLA II peptides exhibited highest 98.46% preserved nature with geographical region I and II, 72.30% with geographical region III, 87.69% with geographical region IV and 87.69% with geographical region V. The extracted HLA I peptides exhibited highest 99.09% preserved nature with geographical region I, 98.18% with geographical region II, 82.72% with geographical region III, 93.63% with geographical region IV and 89.09% with geographical region V. The MCLLANTTF, LLANTTFPC, VNSVAIPLL and VTRLGVNSV peptides were shown binding with both HLA class I and II alleles. LLANTTFPC, LQISVFSTAL, AALILIVVL epitopes of
envelope protein and VNSVAIPLL, VTRLGVNSV, VLLPNVHTL epitopes of nonstructural protein were exhibited complete conservancy with high HLA allele coverage worldwide.

### Peptide Fragments Sharing Both B Cell and T Cell Epitope Property

CHIKV six (NCGGSNEGL, RNEATDGTGL, GTLKIQVSL, LKIQVSLQI, YAVHAPTSLS, RSAEDPERL) T cell peptide epitopes were found in fragment of B cell epitopes but LQISFSTAL and YEKEPEEPTL peptides reveal moderately share with B cell epitope peptide region. Sharing of B cell as well as T cell epitope property in the same fragment of peptides makes epitope dominancy to induce both humeral as well as adaptive immunity (Sette and Fikes 2003).

### Table 1

| S.No | Epitope position | Predicted T cell epitopes | pI value | HLA alleles, class | TAP binding IC50 (nM) | Different geographical region of CHIKV |
|------|------------------|--------------------------|----------|--------------------|-----------------------|---------------------------------------|
| Capsid  
1 | 116–124 | KGRVVAIVL | 11.0 | 38, I | 401.79 | KGRVVAIVL* |
| 2 | 39–47 | IDNADLAKL | 4.21 | 25, I | – | IDNADLAKL* |
| 3 | 49–57 | FKRSSKYDL | 9.7 | 20, II | 172.19 | FKRSSKYDL* |
| E3 Protein  
4 | 2–10 | LLANTTFPC | 5.52 | 47, I | 104.95 | LLANTTFPC* |
| 5 | 21–29 | YEKEPEEPTL | 4.09 | 47, I | 118.58 | YEKEPEEPTL |
| 6 | 2–10 | LLANTTFPC | 5.52 | 46, II | – | LLANTTFPC* |
| E2 Protein  
7 | 31–39 | GTLKIQVSL | 8.75 | 37, I | 2.21 | GTLKIQVSL* |
| 8 | 25–33 | RNEATDGTGL | 4.37 | 19, I | 4325.14 | RNEATDGTGL* |
| 9 | 352–360 | TMTVVVVSVS | 5.19 | 21, I | 33.73 | TMTVVVVSVS |
| 10 | 33–41 | LKIQVSLQ | 8.75 | 31, II | 92.68 | LKIQVSLQI* |
| E1 Protein  
11 | 490–498 | AALILIVVL | 5.57 | 19, I | 2.62 | AALILIVVL* |
| 12 | 418–426 | LQISFSTAL | 5.52 | 18, I | 306.9 | LQISFSTAL* |
| 13 | 274–279 | LILIVVLCV | 5.52 | 29, II | 274.79 | LILIVVLCV* |
| Nsp1 Protein  
14 | 215–223 | VLFSVGSTL | 5.49 | 24, I | 16.11 | VLFSVGSTL* |
| 15 | 130–138 | YAVHAPTSLS | 6.74 | 21, I | 52.84 | YAVHAPTSLS* |
| 16 | 61–69 | RSAEDPERL | 4.68 | 16, I | 366.44 | RSAEDPERL* |
| 17 | 261–269 | YVVKRITMS | 9.99 | 22, II | 722.77 | YVVKRITMS(L5)S* |
| Nsp2 Protein  
18 | 72–80 | FACHSGTLL | 6.73 | 35, I | 937.56 | FACHSGTLL* |
| 19 | 124–132 | KSISRRCTL | 10.86 | 34, I | 2.41 | KSISRRCTL* |
| 20 | 74–82 | CHSGLTVLAL | 6.73 | 15, I | 2648.50 | CHSGLTVLAL* |
| 21 | 43–51 | GLEISARTV | 6 | 14, I | 500.03 | G(N5)LEISARTV |
| 22 | 107–115 | MQMKVNYNH | 8.37 | 16, II | 4.36 | MQMKVNYNH* |
| Nsp3 Protein  
23 | 97–105 | VNSVAIPLL | 5.49 | 37, I | 32.81 | VNSVAIPLL* |
| 24 | 92–100 | VTRLGVNSV | 9.72 | 23, I | 151.36 | VTRLGVNSV* |
| 25 | 92–100 | VTRLGVNSV | 9.72 | 32, II | – | VTRLGVNSV* |
| 26 | 97–105 | VNSVAIPLL | 5.49 | 8, II | – | VNSVAIPLL* |
| Nsp4 Protein  
27 | 187–195 | VLLPNVHTL | 6.71 | 14, I | 23.88 | VLLPNVHTL* |
| 28 | 378–386 | RLFKLGKPL | 11.17 | 13, I | 0.42 | RLFKLGKPL* |
| 29 | 341–349 | VKIIDAVVS | 5.81 | 26, II | 4909.08 | VKIIDAVVS* |

*Showed completely conserve epitope in all geographical regions such as India-1, Italy-2, Senegal-3, Thailand-4 and USA-5
Peptide Fragments Sharing Both B Cell and T Cell Epitope (Green Text)

Seq1: KCNCGSNEGGLTTTDKVVINN protein E2 envelope.
Seq2: ERIRNEATDLKIQVSLQI protein E2 envelope.
Seq3: FPCSQQPCTPCCYEKEPEET protein E3 envelope.
Seq4: REAEIEVEGNSFLQISFSTA protein E1 envelope.
Seq5: KYHCVCPMRSAEDPERLANY NsP1 non structural.
Seq6: IYQDVYAVHAPSLYHQAIK NsP1 non structural.

CHIKV Proteome Extracted Peptides Population Coverage Study

The peptide epitopes LLANTTFPC, LQISFSTAL, AALILIVVL, KGRVVAIVL, VLLPNVHTL, VNSVAILLP and VTRLGVNSV exhibited binding to all HLA allele members of B7, A24, A3, A2 supertype HLA class I (Table 2). Likewise peptide epitopes LLANTTFPC, LRMLEDNVM, LKIQVSLQI, LILIVVLCV, VTRLGVNSV and VNSVAILLP were exhibited binding to almost all allele members of Main DP, DR4, DR3, DR2 class II HLA supertype (Table 3). In this analysis, peptide epitopes were selected which showed their percentile value or range less than 50 but in some case value were found more than 50 percentile value as shown in Table 2. According to analysis less percentile value or range means high affinity with HLA allele. See Table 4.

The above tabulated superantigen peptides LLANTTFPC, LQISFSTAL, AALILIVVL, KGRVVAIVL, VLLPNVHTL, VNSVAILLP and VTRLGVNSV exhibited binding to all HLA allele members of B7, A24, A3, A2 supertype HLA class I (Table 2). Likewise peptide epitopes LLANTTFPC, LRMLEDNVM, LKIQVSLQI, LILIVVLCV, VTRLGVNSV and VNSVAILLP were exhibited binding to almost all allele members of Main DP, DR4, DR3, DR2 class II HLA supertype (Table 3). In this analysis, peptide epitopes were selected which showed their percentile value or range less than 50 but in some case value were found more than 50 percentile value as shown in Table 2. According to analysis less percentile value or range means high affinity with HLA allele. See Table 4.

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Three Dimensional Structure Modelling of all Extracted Peptides

All nanomer extracted potential peptides (LLANTTFPC, LQISFSTAL, AALILIVVL, KGRVVAIVL, VLLPNVHTL, VNSVAILLP, VTRLGVNSV, LKIQVSLQI, and LILIVVLCV) were structurally modelled through PEPstrMOD server. See Fig. 2.

Superantigenic CHIKV Epitopes and HLA Alleles Binding Interaction Study

Binding study of superantigen epitopes LLANTTFPC, LQISFSTAL, AALILIVVL, KGRVVAIVL, VLLPNVHTL, VNSVAILLP, VTRLGVNSV, LKIQVSLQI, and LILIVVLCV with HLA supertype favoured alleles, were done for analyse the interaction of both peptide and allele molecules through Autodock 4.2. During the binding interaction study, the two peptides and allele docked complexes such as LLANTTFPC–B*0702 allele and LQISFSTAL–A*0301 allele exhibited best binding energy of −3.13 and −3.19 kcal/Mol, among all experiments respectively. Peptide LLANTTFPC–B*0702 allele complex appeared with one TRY27:HH Hydrogen bond (Fig. 3). Similarly peptide VNSVAILLP–A*0301 allele complex appeared with one H bond viz. ARG6:HH12 (Fig. 4).

Docking of Top Extracted Class I HLA Binding Peptides with cTap Protein

As the above study revealed extracted several peptides which represent a good binding with HLA class I alleles. These all peptides under gone for antigen processing and presentation confirmation by docking with cTap protein. cTap protein is crucial protein during antigen processing and presentation of class I HLA binding peptides. cTAP channelized and facilitated the peptides passage throughout the transportation from cytoplasm to Endoplasmic reticulum (ER) and provide succesful presentation over the cell membrane to make interaction with T cell receptors. So, the peptides and cTAP interaction also be a major factor to confirm the peptide potential as vaccine candidacy. During this study, these two LLANTTFPC and VNSVAILLP peptides showed optimum cTAP protein binding pattern with with −1.00 and −1.37 kcal/Mol.
Table 2  Top CHIKV peptide epitopes with high affinity with HLA Class I Supertypes allele members by ANN and SMM algorithms of IEDB platform. (Under 50 percentile value show high affinity with allele)

| S.No | Epitope                              | A24 Supertype (Percentile value) | A23 Supertype (PercentileValue) | A2 Supertype (PercentileValue) | A3 Supertype (PercentileValue) |
|------|--------------------------------------|----------------------------------|---------------------------------|--------------------------------|--------------------------------|
|      |                                      | A*2407  | A*2405  | A*2403  | A*2402  | A*2301  | A*6801  | A*3301  | A*3101  | A*1101  | A*0301  |
| 1    | STLPETTVV (HBV) + C                  | 5.2     | 6.0     | 6.6     | 6.0     | 5.9     | 2.3      | 2.5      | 0.44     | 0.62     | 0.02     |
| 2    | ILRGSVAHK (H1N1) + C                 | 18      | 21      | 22      | 22      |         | 9.6      | 8.9      | 9.5      | 6.4      | 7.4      |
| 3    | LLANTTFPC                            | 35      | 36      | 25      | 16.5    | 25      | 32.5     | 34.5     | 29       | 17       | 15.5     |
| 4    | KGRVVAIWL                            | 7.1     | 11      | 19.5    | 42      |         | 48.5     | 51       | 19.5     | 30       | 32       |
| 5    | LQIFSSTAL                            | 4.5     | 5.4     | 15.35   | 11.8    | 17      | 49       | 39       | 16.5     | 26       | 31.5     |
| 6    | AALILIVVL                            | 6.2     | 11      | 5.75    | 23      |         | 49       | 39       | 16.5     | 26       | 31.5     |
| 7    | VLLPNVHTL                            | 0.2     | 0.29    | 4.59    | 4.35    |         | 57       | 49.5     | 21       | 28       | 20.5     |
| 8    | VNSVAIPLL                            | 3.2     | 4.4     | 14.55   | 13.25   |         | 50       | 43.5     | 41       | 46       | 42.5     |
| 9    | VTRLGVNSV                            | 8.4     | 12      | 27.5    | 44      |         | 65.5     | 34.5     | 29.5     | 35       | 31.5     |
|      |                                      |         |         |         |         |         | 9.6      | 8.9      | 9.5      | 6.4      | 7.4      |
|      |                                      |         |         |         |         |         | 32.5     | 34.5     | 29       | 17       | 15.5     |
|      |                                      |         |         |         |         |         | 48.5     | 51       | 19.5     | 30       | 32       |
|      |                                      |         |         |         |         |         | 49       | 39       | 16.5     | 26       | 31.5     |
|      |                                      |         |         |         |         |         | 49       | 39       | 16.5     | 26       | 31.5     |
|      |                                      |         |         |         |         |         | 57       | 49.5     | 21       | 28       | 20.5     |
|      |                                      |         |         |         |         |         | 50       | 43.5     | 41       | 46       | 42.5     |
|      |                                      |         |         |         |         |         | 65.5     | 34.5     | 29.5     | 35       | 31.5     |
|      |                                      |         |         |         |         |         | 9.6      | 8.9      | 9.5      | 6.4      | 7.4      |
|      |                                      |         |         |         |         |         | 32.5     | 34.5     | 29       | 17       | 15.5     |
|      |                                      |         |         |         |         |         | 48.5     | 51       | 19.5     | 30       | 32       |
|      |                                      |         |         |         |         |         | 49       | 39       | 16.5     | 26       | 31.5     |
|      |                                      |         |         |         |         |         | 49       | 39       | 16.5     | 26       | 31.5     |
|      |                                      |         |         |         |         |         | 57       | 49.5     | 21       | 28       | 20.5     |
|      |                                      |         |         |         |         |         | 50       | 43.5     | 41       | 46       | 42.5     |
|      |                                      |         |         |         |         |         | 65.5     | 34.5     | 29.5     | 35       | 31.5     |

|      |                                      | A*0206  | A*0205  | A*0203  | A*0204  | A*0301  | A*5102  | B*5301  | B*5101  | B*3501  | B*0702  |
| 1    | STLPETTVV (HBV) + C                  | 0.2     | 0.37    | 1.1     | 1.8     |         | 0.57    | 3.3     | 0.84    | 4.2     | 3.4     |
| 2    | ILRGSVAHK (H1N1) + C                 | 16      | 12      | 6.8     | 12      |         | 54      | 43      | 51      | 30      | 14      |
| 3    | LLANTTFPC                            | 3.71    | 5.3     | 5.85    | 5       |         | 13      | 34      | 25      | 19      | 28      |
| 4    | KGRVVAIWL                            | 30      | 14      | 44      | 39      |         | 59      | 6.6     | 41      | 48      | 4.3     |
| 5    | LQIFSSTAL                            | 0.34    | 0.18    | 1.99    | 1.8     |         | 5.4     | 3.7     | 12      | 5.5     | 10      |
| 6    | AALILIVVL                            | 11.05   | 17      | 23.5    | 19.25   |         | 12      | 2.3     | 5.5     | 6.7     | 9.4     |
| 7    | VLLPNVHTL                            | 1.62    | 0.03    | 1.6     | 2.7     |         | 35      | 0.89    | 15      | 26      | 13      |
| 8    | VNSVAIPLL                            | 37.5    | 5.3     | 39      | 22.5    |         | 24      | 4.7     | 11      | 52      | 26      |
| 9    | VTRLGVNSV                            | 10.9    | 1.6     | 3.75    | 25.5    |         | 75      | 1.3     | 13      | 74      | 4.1     |

|      |                                      |         |         |         |         |         | 2.3      | 3.4      | 3.8      |         |         |
|      |                                      |         |         |         |         |         | 9.6      | 5.7      | 20       |         |         |

|      |                                      |         |         |         |         |         | 2.3      | 3.4      | 3.8      |         |         |
|      |                                      |         |         |         |         |         | 9.6      | 5.7      | 20       |         |         |
### Table 2 (continued)

**CHIKV HLA Class I Supertype analysis**

| S.No | Epitope            | A*2407 (Percentile value) | A*2405 | A*2403 | A*2402 | A*2301 |
|------|--------------------|---------------------------|--------|--------|--------|--------|
| 3    | LLANTTFPC          | 17.35                     | 8.5    | 11.5   |        |        |
| 4    | KGRVVAIVL          | 32.5                      | 15     | 42.5   |        |        |
| 5    | LQISFSTAL          | 4.38                      | 0.3    | 17     |        |        |
| 6    | AALILIVVL          | 27.5                      | 12     | 29     |        |        |
| 7    | VLLPNVHTL          | 4.4                       | 14     | 23.5   |        |        |
| 8    | VNSVAILPLL         | 27.45                     | 38     | 30     |        |        |
| 9    | VTRLGCVNSV         | 49.5                      | 20     | 9.2    |        |        |

### Table 3

**Top CHIKV peptide epitopes with high affinity with HLA Class II Supertypes allele members by ANN and SMM algorithms of IEDB platform. (Under 50 percentile range show high affinity with allele)**

**CHIKV HLA Class II Supertype analysis**

| S.No | Epitope            | DRB1*1501 | *1101 | *0901 | *0701 | *0101 |
|------|--------------------|-----------|-------|-------|-------|-------|
| 1    | ILRGSVAHK (H1N1)  | 6.80–44   | 18–32 | 24–48 | 11–36 | 4.80–27 |
| 2    | STLPETTVV (HBV)   | 50–92     | 53–67 | 20–98 | 34–76 | 15–90 |
| 3    | LLANTTFPC         | 61–84     | 41–73 | 77–88 | 33–70 | 38–82 |
| 4    | LRMLEDNVNM        | 31–38     | 56–73 | 49–61 | 71–86 | 34–57 |
| 5    | LKIQVSLQI         | 2.40–6.80 | 20–29 | 12–26 | 2.5–5.90 | 17–36 |
| 6    | LILIVLCV          | 3.60–4.10 | 13–22 | 61     | 8.20–15 | 11–18 |
| 7    | VTRLGCVNSV        | 34–38     | 22–62 | 14–38 | 9.20–38 | 15–18 |
| 8    | VNSVAILPLL        | 17–34     | 44–62 | 14–22 | 9.20–32 | 15–23 |

**Supertype DR4**

| S.No | Epitope            | DRB1*0802 | *0405 | *0901 | *0401 | DRB3*0101 | *0101 |
|------|--------------------|-----------|-------|-------|-------|-----------|-------|
| 1    | STLPETTVV (HBV)   | 25–83     | 33–83 | 23–76 | 71–82 | 19–59     |       |
| 2    | ILRGSVAHK (H1N1)  | 6.40–21   | 32–72 | 6.90–28 | 65–76 | 29–56     |       |
| 3    | LLANTTFPC         | 39–75     | 38–80 | 21–67 | 24–59 | 42–82     |       |
| 4    | LRMLEDNVNM        | 54–74     | 18–54 | 13–27 | 7.60–33 | 70–56     |       |
| 5    | LKIQVSLQI         | 5.10–36   | 16–31 | 2.10–20 | 27–35 | 16–49     |       |
| 6    | LILIVLCV          | 3.30–13   | 2.10–7.30 | 7.60–28 | 67    | 20–28     |       |
| 7    | VTRLGCVNSV        | 13–35     | 15–44 | 22–23 | 36–81 | 30–65     |       |
| 8    | VNSVAILPLL        | 29–38     | 19–52 | 22–47 | 36–73 | 30–73     |       |

**Supertype DP2**

| S.No | Epitope            | B1*0201 | B1*0101 | B1*0402 | B1*0501 |
|------|--------------------|---------|---------|---------|---------|
| 1    | ILRGSVAHK (H1N1)  | 59–72   | 19–53   | 24–56   | 4.70–25 |
| 2    | STLPETTVV (HBV)   | 38–72   | 17–77   | 26–79   | 35–65   |
| 3    | LLANTTFPC         | 13–35   | 43–69   | 29–57   | 55–77   |
| 4    | LRMLEDNVNM        | 76–81   | 40–69   | 55–62   | 37–58   |
| 5    | LKIQVSLQI         | 25–56   | 13–32   | 16–38   | 7.50–32 |
| 6    | LILIVLCV          | 14–34   | 58–86   | 38–67   | 50–87   |
| 7    | VTRLGCVNSV        | 23–76   | 21–43   | 29–52   | 30–35   |
| 8    | VNSVAILPLL        | 23–32   | 19–30   | 29–52   | 29–38   |
binding energy, respectively (Saxena and Mishra 2020). See Figs. 5 and 6.

**The Allele and Epitope Complexes, Physical Interaction Study by MD Simulation**

Complexes 2LLANTTFPC\textsuperscript{10}–B*0702 allele and 98VNSVAIPLL\textsuperscript{106}–A*0301 allele were studied for their physical interactions within in time window through NAnoscale Molecular Dynamics (NAMD) simulation. During whole simulation process the RMSD values were stored in RMSD. DAT file with respect to time frame and RMSD graph was obtained. During the simulation RMSD graph initially rise up continuously with variations which occurred during interaction between epitope- HLA complex with change in time. Complexes 2LLANTTFPC\textsuperscript{10}–B*0702 allele during simulation observed highest RMSD value 10.4 Å at 6200 picoseconds with stable interaction pattern. Similarly 98VNSVAIPLL\textsuperscript{106}–A*0301 allele complex also observed with stable physical interaction with highest RMSD value 7.7 Å at 8200 picoseconds. This molecular simulation of protein–protein interaction will show physical stable bonding patterns and their motion during interaction which confirm conserve peptide and allele stable interaction.

**Discussion**

The present hierarchical approach for finding of finest T cell peptide epitopes as most promising candidates for CHIKV vaccine divided in two phase study (i) Sequence based screening of peptides through propred and IEDB Server (ii) Structure based study through docking and simulation analysis. During the study peptides LLANTTFPC of structural envelope protein E3 and VNSVAIPLL of non structural protein nsp3 were observed most prominent over all consider parameters such as peptide conserve nature, supertype population coverage among all extracted peptides from whole proteome. The envelope E1 glycoprotein of virus helps in smooth access of the viral protein into the host cell and the non-structural nsp3 protein of Chikungunya known to function as a viral RNA transcription regulator (Saxena and Mishra 2020). Both the protein have specific prominent role in the virus life cycle and also presents the TAP protein binding efficiency during sequence based analysis. As already known, the peptides and TAP interaction also be a major factor in antigen processing and their presentation over Class I HLA molecule (Mishra et al. 2020). The first phase screening and supertype analysis will result as top two peptides LLANTTFPC of protein E3 and VNSVAIPLL of protein nsp3 as superantigen peptides which observed as a common binder for class I and class II HLA supertype all allele members (Table 2 and Table 3). Peptide binding with allele members shown good results as IEDB recommended values. The peptide epitopes LLANTTFPC, LQISFSTAL, AALILIVVL, KGRVVAIYL, VLLPNVHTL, VNSVAIPLL and VTRLGVNSV exhibited binding to all HLA allele members of B7, A24, A3, A2 supertype HLA class I (Table 2). Likewise peptide epitopes LLANTTFPC, LRMLEDNVMT, LKIQLSGLQ, LILIVVLCV, VTRLGVNSV and VNSVAIPLL exhibited binding to almost all allele members of Main DP, DR4, DR3, DR2 class II HLA supertype (Table 3). Overall the peptide LLANTTFPC and VNSVAIPLL were observed more promising than other as vaccine candidacy. For further confirmation it is necessary to

| S.No | Peptide   | HLA Allele     | Autodock4.2(kcal/Mol) |
|------|-----------|----------------|-----------------------|
|      |           |                | BE        IME         IE     TorE   H Bond |
| 1    | LLANTTFPC | B*0702         | −3.13     −11.18     −7.44   + 8.05  1 |
| 2    | LLANTTFPC | B*5301         | −2.18     −10.23     −5.26   + 8.05  1 |
| 3    | LLANTTFPC | A*0301         | −1.71     −9.77      −5.96   + 8.05  1 |
| 4    | LLANTTFPC | B*3501         | 1.69      −9.75      −5.49   + 8.05  1 |
| 5    | LLANTTFPC | B*5101         | −1.37     −9.42      −5.52   + 8.05  2 |
| 6    | LLANTTFPC | A*0101         | −1.26     −9.77      −5.96   + 8.05  1 |
| 8    | LLANTTFPC | DRB 0401       | −2.68     −10.73     −4.83   + 8.05  1 |
| 9    | VNSVAIPLL | A*0301         | −3.19     −11.24     −4.35   + 8.05  1 |
| 10   | VNSVAIPLL | A*0101         | −2.97     −11.02     −4.20   + 8.05  2 |
| 11   | VNSVAIPLL | B*3501         | −2.43     −10.48     −3.97   + 8.05  2 |
| 12   | VNSVAIPLL | B*5101         | −1.99     −10.05     −4.43   + 8.05  1 |
| 13   | VNSVAIPLL | B*5301         | −1.32     −9.38      −3.99   + 8.05  1 |
| 14   | VNSVAIPLL | B*0702         | −1.50     −9.55      −4.52   + 8.05  1 |
| 15   | VNSVAIPLL | DRB1 0101      | −3.01     −11.07     −3.91   + 8.05  1 |
| 16   | VNSVAIPLL | DRB1 0301      | −2.76     −10.82     −4.15   + 8.05  0 |
Fig. 1 Workflow of computational proteome exploration for finding of HLA and cTAP binding novel epitopes as CHIKV vaccine candidates

- Genome & all protein sequences of CHIKV were retrieved from NCBI database
- Study of physiological properties of all CHIKV viral proteins was done by Expasy
- Prediction of HLA I & HLA II binding Epitopes by Propred I and Propred, respectively
- Cross check of screened epitope with other algorithms viz. IDEB (ANN etc)
- All the predicted epitope of CHIKV protein were subjected to worldwide conserve region analysis among all geographical regions (IEDB conservancy tool)
- pi value analysis of each mutated epitope by protparam
- B cell epitope screening of CHIKV protein by BCPred and AAP algorithms
- Selection and analysis for finding the T cell epitopes which are sharing the B cell epitope fragment
- Supertype analysis for finding of superantigen properties of predicted peptide HLA I and II by IEDB platform
- Above several folds screening of epitopes will reduce the promiscuous epitopes numbers on the basis of sequence based methods
- Completely conserved, super antigenic and maximum HLA binding peptides were selected as potential epitope for structure based analysis for their validation as vaccine candidates
- Above epitopes and respective HLA molecules were modeled in 3D structures and epitopes interactions to cTAP and HLA were examined by docking analysis to confirm antigen processing and presentation
- Epitope and HLA molecule complexes showed their good binding through docking analysis were further validated by molecular dynamic simulation study to confirm the stability of their complex to generate immune response
- Finally, the above methodology will give potential conserve superantigenic peptide epitopes. Those epitopes can be further validated by wet lab experiments to check their CHIKV vaccine candidacy as well as rapid diagnostics reagents
analyze these peptides for their structure-based binding simulation study to confirm their physical interaction to concern HLA allele as well as cTap protein.

Binding study of superantigen epitopes $^2$LLANTTFPC$^{10}$ and $^9$VNSVAIPLL$^{106}$ with HLA supertype favored alleles, were done through Autodock 4.2. During the binding interaction study, the two peptide and allele docked complexes such as $^2$LLANTTFPC$^{10}$–B*0702 allele and $^9$VNSVAIPLL$^{106}$–A*0301 allele exhibited best binding energy of $-3.13$ kcal/Mol with one TRY27:HH Hydrogen bond and $-3.19$ kcal/Mol with one H bond viz. ARG6:HH12, among all experiments, respectively. Further, these complexes stability confirmation done by using simulation NAMD- VMD platform. Complexes $^2$LLANTTFPC$^{10}$–B*0702 allele during simulation observed highest RMSD value 10.4 Å at 6200 picoseconds with stable interaction pattern (Fig. 7). Similarly $^9$VNSVAIPLL$^{106}$–A*0301 allele complex also observed with stable physical interaction with highest RMSD value 7.7 Å at 8200 picoseconds (Fig. 8). This molecular simulation of protein–protein interaction will show physical stable bonding patterns and their motion during interaction which confirm conserve peptide and allele stable interaction.

As both peptides showed binding with class I HLA molecule means these peptides processing and presentation depended on interaction with cTAP protein. Protein cTAP channelized and facilitated the peptides passage throughout the transportation from cytoplasm to Endoplasmic reticulum (ER) and provide successful presentation over the cell membrane to make interaction with T cell receptors. So the peptides and cTap interaction also be a major factor to confirm the peptide potential as vaccine candidacy which will be done by docking of both peptides to cTap protein (Sharma et al. 2018). Peptide LLANTTFPC exhibited two hydrogen bonds with cTAP1 cavity viz. SER545 and GLU587 with $-1.00$ kcal/mol binding energy. Peptide VNSVAIPLL exhibited three hydrogen bond 1 with cTAP1 cavity viz. SER545 and
GLU587 with −1.37 kcal/Mol binding energy (Sharma et al. 2017; Saxena and Mishra 2020). Both peptides showed optimum binding in cTAP protein cavity, which confirm as a channel passageway to peptide transport through the cytoplasm to lumen of ER. Apart from these two peptides, many potential epitopes shown good results which can employ for diagnostic purpose with use of vaccine candidacy. As reported earlier, that both structural and non-structural proteins presently involved in design and development the peptide-based vaccine for Dengue and Hepatitis (Kaushik et al. 2014; Henrique et al. 2013), even in fungal and veterinary vaccine development also reported. Recently, several latest researches have been reported, which encourage the Immunoinformatics top down approach to get superantigenic epitopes from whole proteome of viral, bacterial, fungi (Sharma et al. 2019; Joshi and Kaushik 2020; Akhtar et al. 2021) or many more infectious agents to design diagnosis reagents and vaccines against them. Although, computational studies now these days having a major role in research in the way to reduce experimental cost and time, still these computational studies having some limitation to apply them directly to the natural world (Krishnan et al. 2020). To remove this barrier identified products epitopes should be further tested in a wet laboratory as diagnosis purpose as well as vaccine candidacy for Chikungunya.

Fig. 5 A The docking interaction of peptide LLANTTFPC with grey solid phase and cTAP1 (IJJ7 PDB) with white solid phase showed channelling in cTap cavity and facilitated the peptides passage throughout the transportation from cytoplasm to Endoplasmic reticulum (ER). B Peptide LLANTTFPC exhibited two hydrogen bonds with cTAP1 cavity viz. SER545:THR6 and GLU587:CYS9 with −1.00 kcal/Mol binding energy

Fig. 6 A The docking interaction of peptide VNSVAIPLL with grey solid phase and cTAP1 (IJJ7 PDB) with white solid phase showed channelling in cTap cavity and facilitated the peptides passage throughout the transportation from cytoplasm to Endoplasmic reticulum (ER). B Peptide VNSVAIPLL exhibited three hydrogen bonds with cTAP1 cavity viz. SER545:VAL4, SER545:ILE6 and GLU587:LEU9 with −1.37 kcal/Mol binding energy
proteome of CHIKV can be a novel step in development of CHIKV epitope based vaccine as well as diagnostic development with aspect of time, cost and side effects.

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Declarations

Conflict of interest The authors declare that they have no conflict of interest.

Ethical Approval This article does not contain any studies with human participants or animals performed by any of the authors.

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Fig. 7 NAMD-VMD simulation- RMSD graph with time window showed physical interaction of 2LLANTTFPC10–B*0702 allele complex during the simulation which observed highest RMSD value 10.4 Å at 6200 picoseconds with stable interaction pattern

Fig. 8 NAMD-VMD simulation- RMSD graph with time window showed physical interaction of 98VNSVAIPLL106–A*0301 allele complex during the simulation also observed with stable physical interaction with highest RMSD value 7.7 Å at 8200 picoseconds similar hierarchical approach also done for many infectious viruses such as Dengue, H1N1, JEV, West Nile virus and tuberculosis gave most promising results in vaccine development research (Jardine et al. 2013; De Groote et al. 2013; Feng et al. 2013; Khan et al. 2019; Sharma and Kumar 2010).

 Conclusion

As development of tools and techniques in immunoinformatics top down approach, made easier and feasible to surmount the burden of medical health system by design and development of epitope based vaccine against severe infections like Chikungunya, H1N1, JEV etc. The present analysis, peptides 2LLANTTFPC10 of protein E3 and 98VNSVAIPLL106 of protein nsP3 were observed most superantigenic with consideration of all parameters such as peptide conserve nature, supertype population coverage, tap binding among all extracted peptides from whole proteome. Overall, this multistep screening and crosscheck structural binding analysis with exploration of complete proteome of CHIKV can be a novel step in development of CHIKV epitope based vaccine as well as diagnostic development with aspect of time, cost and side effects. We are thankful to Prof. Sheeba, Dr. B.R. Ambedkar University, Agra, India for his experimental guidance during the course of experimental work. We are also indebted to Prof. Ajay Kumar, Department of Biotechnology, Faculty of Engineering and Technology, Rama University, Kanpur, India for giving us necessary suggestions and inspiration to successfully accomplish the write-up of this manuscript.

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