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How to Cite

Ahmad I, Khan H, Salman M, Rehman L, Khan SA, Saddam, Khan K, Naeem I, Qazi NU. Modelling interaction between non-structural protein 2 of Chikungunya Virus and various protein factors of innate pathway. Biomedical Letters 2022; 8(2):162-169.

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Modeling interaction between non-structural protein 2 of Chikungunya Virus and various protein factors of innate pathway

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Abstract

Chikungunya virus is positive-sense single-stranded RNA virus that causes an arthropod-borne chikungunya fever, myalgia and arthralgia. Chikungunya virus belongs to the *Togaviridae* family, and the genus is *Alphavirus*. Virus-host protein interaction plays a vital role in developing vaccines and antiviral drugs. We designed the current study to establish the *in-silico* interaction of non-structural protein 2 (nsP2) with proteins of innate immune pathway. The nsP2 sequences of various Chikungunya virus genotypes were retrieved from National Centre for Biotechnology Institute (NCBI). The homology models of proteins were generated through a protein modeling online web server. Protein-protein interaction (PPI) between nsP2 and proteins of innate immune pathway were docked using High Ambiguity-Driven Docking (HADDOCK) webserver. The interactive residues of the bimolecular complexes were analyzed with PDBsum-Generate online webserver. Our findings revealed differentially affinity of nsP2 of various chikungunya genotypes towards key proteins of cellular innate pathway. The nsP2 of Asian genotype demonstrates relatively high interaction with interferon-beta promoter stimulator 1 (IPS-1). Similarly, nsP2 of various genotypes binds with differential affinity to tumor necrosis factor receptor-associated factor 6 (TRAF6) with the highest affinity observed for the nsP2 of the West African genotype. Bimolecular complexes of nsP2 and host proteins demonstrate the interaction of various domains of nsP2 with proteins of the innate immune pathway. Thus, it is sought that the selected panel of the proteins might be helpful to treat the viral infection as a therapeutic drug target in the future.

Introduction

Chikungunya virus (CHIKV) is an arthropod-borne human pathogen causing chikungunya fever associated with arthralgia, myalgia, headache, skin rash, and joint swelling [1]. In1953, CHIKV was a first time isolated from a patient in Tanzania [2]. CHIKV has three genotypes: Asian, West African (WA), and East Southern African (ECSA) [3]. CHIKV belongs to the family *Togaviridae and the* genus *alphavirus*. CHIKV is an enveloped single-stranded positive-sense RNA virus having a 5`methylation cap and 3`polyadenylation tail. CHIKV genome comprises two open reading frames that encode two polyproteins and subsequently split into three structural proteins; capsid (C) and envelop glycoproteins (E1 and E2) that together facilitate viral replication in the host cell. In contrast, the four non-structural proteins (nsP1-4) are most significant for RNA replication because of their enzymatic functions [4,5]. The nsP1 regulates RNA capping, nsP2 has helicase and protease activity, nsP3 is involved in RNA replication, and nsP4 facilitates RNA-dependent RNA polymerase functions as shown in (**Fig. 1**) [4,6].

Among the CHIKV proteins, nsP2 is responsible for the host transcriptional shut-off and facilitates viral replication [7]. The nsP2 is five domain long-chain protein consisting of 798 amino acid (aa) residues. The N-terminal site of nsP2 encodes three domains of (1-167 aa residues), while two Rec A domains encode

with (168-470 aa residues). Rec A domain is responsible for nucleoside triphosphatase and RNA-dependent triphosphatase functions [8]. While the C-terminal site has two structural domains; the protein-like protease (PLP) domain encodes (471-605 aa residues), and the methyl transferase-like domain (MTL) encodes (606-798 aa residues) respectively. The MTL domain is considered non-functional due to the lack of significant structural elements. On the other hand, the PLP domain has a proteolytic function that processes polyproteins and is required for viral replication [9]. The CHIKV nsP2 receptor binding domain is an ideal site to be the target in the drug development pipeline.[10].

The current study involves implementing bioinformatics tools to predict protein-protein interaction of CHIKV nsP2 and human innate pathways proteins. The bioinformatics approach relies on the protein structure information to determine protein interaction with their target proteins [11]. Previously, researchers used various bioinformatics tools for predicting virus-host interactions for obtaining information from protein structure [12,13]. Researchers currently using sequence and homology models to identify virus-host endogenous and exogenous protein interactions [14]. Recently, the incredible developments in bioinformatics tools make it possible that protein-protein interaction enhances the innovation of many potential inhibitors against the pathogen [15].
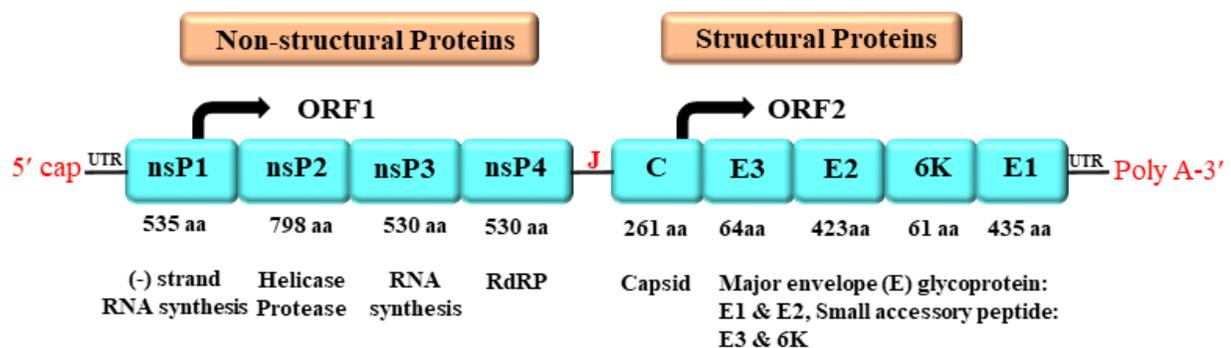
Fig. 1: Organization of Chikungunya virus whole genome. The figure shows both nonstructural, and structural proteins, and 5′ and3′ untranslated regions

Recently, the re-emergence of arboviruses, including CHIKV has raised public health concerns because of the virus's contagious nature [2]. The arboviral diseases have caused large-scale outbreaks in the last twenty years, specifically in an endemic country like Brazil, which has resulted in high rates of mortality and morbidities. Similarly, the emergence of SARS-

CoV-2 in these arboviral endemic areas was initially misdiagnosed because of the coinfection with either CHIKV or dengue [16]. There is no well-developed controlling and management strategy existing for individuals suffering post-CHIKV consequences for many years. This neglect might be because both CHIKV and dengue have been considered a simple

and short-lived infection [17]. Therefore, it is more desirable to establish controlling and therapeutic strategies against CHIKV.

Materials and Methods

Protein sequences retrieval and modeling

We retrieved nsP2 sequences of CHIKV genotypes; Asian, WA, ECSA, and human innate immune pathway proteins from the NCBI. Through SWISS-MODEL webserver (https://swissmodel.expasy.org/interactive), we modeled the Three-dimensional (3D) structure of CHIKV nsP2 and proteins of the human innate pathway such as; retinoic acid-inducible gene I (RIG-I), interferon-beta promoter stimulator 1 (IPS-1), Tumor necrosis factor receptor-associated factor 3 (TRAF3), Tumor necrosis factor receptor-associated factor 6 (TRAF6), and TIR domain-containing adaptor inducing interferon-β (TRIF).SWISS-MODEL first aligns the amino acid sequence of the target protein and uses this sequence as a query against the template library. Further select the best template on the base of sequence identity, estimated quaternary structure, and global model quality estimate then generates a 3D structure of the protein. The stereochemical stability of the modeled proteins was analyzed through the ERRAT program. The higher ERRAT scores represent good qualities of protein. Generally, a score above 50 was considered a good quality model [18]. The ERRAT scores of both virus and host were shown in **Table S1**.

Docking of nsP2 and Proteins of Innate Immune Pathway

The energy of proteins was minimized with molecular operating environment (MOE) software, using the Amber 10 force field by adding missing hydrogen and partial charge to proteins [11]. To gain insight into protein interaction, we docked nsP2 and innate immune proteins through an online web server, High Ambiguity Driven protein-protein DOCKing (HADDOCK), and pyDockWEB [19, 20,21].

Analysis of biomolecular complex and interacting residues

We analyzed the bimolecular complex using UCSF Chimera (version 1.13.1) [22]. The PDBsum-Generate(http://www.ebi.ac.uk/thornton-srv/databases/pdmsum/Generate.html) online tool was used and determined various kinds of interactions between the interactive residues of virus-host proteins.

Results

HADDOCK webserver provides the top ten biomolecular complexes. We selected the best complex based on the HADDOCK score, electrostatic, and Van der Waals energies. HADDOCK score is the average of all given energies [23]. The statistical scores of the HADDOCK complexes were shown in **Table 1**. Best complexes have the lowest electrostatic, van der Waals energies, and HADDOCK score. The stability of two proteins is based on the number of active residues involved in the complex formation and shows the interface between the two molecules. Large interfaces between the molecules show high binding energies [23]. We also used the pyDockWEB server to validate the HADDOCK complexes. pyDockWEB provides both protein-protein/ligand interaction to the user. pyDockWEB generated several complexes [20]. The best complex was selected through their given energies such as electrostatic, desolvation Vander wall, and total energy, as shown in **Table S2**.

The sequence alignment data shows that CHIKV nsP2 was highly conserved within genotypes. There were very few mutations among all genotypes as shown in (**Fig. 2**). The PDBsum-Generate results showed various kinds of interaction involved in the stability of protein-protein interaction. CHIKV nsP2 showed various numbers of hydrogen bonds, and salt bridge interaction for either genotype-specific/host, as we described in **Table S3-S7 and Fig. S2-S7**.

HADDOCK complexes have various levels of interaction affinity between nsP2 of different CHIKV genotypes and various cellular proteins of the innate pathway. RIG-I senses single-stranded RNA viruses to induce antiviral responses. In the current study, RIG-1 was included as a positive control to determine protein-protein interaction among CHIKV nsP2 and various innate immune proteins. The HADDOCK score of RIG-1 and CHIKV genotypes, Asian, WA, and ECSA, were -103.7 ± 8.4, -105.7 ± 7.2, and -108.5 ± 2.3. HADDOCK score of the biomolecular complexes of nsP2 of CHIKV all genotypes and the human innate immune proteins has comparable binding affinity with the RIG-1 were described in **Table 1** and complexes were shown in **Fig. S1A**. The pyDock complexes score were provided in supplementary material as described in **Table S2**.

Table 1: Analysis of HADDOCK complexes of nsP2 of CHIKV genotypes and human innate immune proteins

CHIKV genotypes -host proteins	HADDOCK Score (a.u)	Van der Waals energy (Kcal/mol)	Electrostatic energy (Kcal/mol)	Desolvation energy (Kcal/mol)	Restraints violation energy (Kcal/mol)	Z-Score
Asian- nsP2- RIG-1	-103.7 ± 8.4	-86.4 ± 7.0	-260.2 ± 44.8	-26.4 ± 5.2	611.4 ± 69.95	-1.5
WA- nsP2- RIG-1	-105.7 ± 7.2	-91.2 ± 4.6	-364.4 ± 37.0	-9.1 ± 2.9	675.4 ± 113.62	-1.3
ECSA-nsP2- RIG-1	-108.5 ± 2.3	-85.6 ± 4.6	-392.8 ± 35.4	-8.1 ± 7.4	637.2 ± 59.58	-1.6
Asian- nsP2- IPS-1	-120.1 ± 4.4	-89.1 ± 4.3	-332.6 ± 49.4	-23.6 ± 2.9	591.2 ± 2.86	-1.7
WA- nsP2- IPS-1	-101.0 ± 6.1	-75.3 ± 15.7	-370.6 ± 88.7	-95.8 ± 13.8	1441.5 ± 103.17	-2.1
ECSA- nsP2- IPS-1	-114.3 ± 7.9	-73.5 ± 8.4	-345.2 ± 16.0	-33.6 ± 1.8	618.6 ± 111.82	-1.7
Asian- nsP2- TRAF3	-102.9 ± 7.4	-89.5 ± 2.8	-352.5 ± 56.1	-36.1 ± 7.8	931.8 ± 109.17	-1.5
WA- nsP2- TRAF3	-110.1 ± 16.2	-97.3 ± 1.7	-377.4 ± 22.9	-9.6 ± 8.0	722.7 ± 113.14	-1.9
ECSA- nsP2- TRAF3	-113.3 ± 4.5	-89.1 ± 6.2	-338.9 ± 57.9	-38.4 ± 3.9	819.8 ± 167.76	-1.9
Asian- nsP2- TRAF6	-102.1 ± 4.3	-72.9 ± 6.4	-419.9 ± 81.7	-0.6 ± 9.5	553.7 ± 44.49	-1.2
WA- nsP2- TRAF6	-93.9 ± 8.1	-71.6 ± 15.0	-302.3 ± 55.6	-24.1 ± 8.2	622.1 ± 71.88	-1.6
ECSA- nsP2- TRAF6	-107.4 ± 2.1	-71.3 ± 4.9	-437.9 ± 46.7	-0.6 ± 8.8	521.5 ± 27.50	-1.4
Asian- nsP2- TRIF	-117.6 ± 5.6	-89.5 ± 2.8	-218.9 ± 45.7	-31.0 ± 8.8	467.7 ± 124.48	-1.5
WA- nsP2- TRIF	112.6 ± 6.9	-91.5 ± 4.5	-212.1 ± 42.3	-35.9 ± 7.8	572.6 ± 47.01	-1.9
ECSA- nsP2- TRIF	-113.5 ± 6.6	-95.0 ± 2.4	-322.2 ± 61.2	-19.0 ± 6.6	649.4 ± 18.45	-1.3

Note: *arbitrary unit (a.u), Kilocalories per mole (kcal/mole), HADDOCK is the sum of all energies involved in protein-protein interaction, Z-score is the standard deviation of the energies*

Among the HADDOCK complexes, the nsP2 CHIKV Asian genotype with IPS-1 showed comparatively high binding affinity while nsP2 of WA genotype with TRAF6 showed low binding affinity -93.9 ± 8.1. Both CHIKV nsP2 and host innate immune pathway proteins were labeled with different colors for better understanding. In the HADDOCK complex, host cellular proteins were presented by green and CHIKV nsP2 in red and blue colors. The red color represents the PLP domain, while the blue represents the MLT domain of nsP2 of CHIKV (**Fig. S1 A-E**).

In the biomolecular complexes chain, A represents CHIKV nsP2 and chain B represents host immune protein. The human host innate immune proteins showed various interactions among CHIKV genotypes, such as RIG-I and nsP2 of Asian genotypes with one disulfide bond, 15 hydrogen bonds, and 189 non-covalent bonds. Similarly, the WA genotype shows one disulfide, 16 hydrogens, and 213 non-covalent bonds, whereas the ECSA genotype shows one disulfide, 21 hydrogen, and 230 nonbonding interactions. The biomolecular complex of IPS-1 and CHIKV genotypes; Asian, WA, and ECSA showed 4, 2, 2 disulfide bonds, 16, 14, 16 hydrogen bonds, and 149, 184, 180 non-bonding interactions, while CHIKV nsP2 -TRAF3 complex showed 4, 3, 2 disulfide bonds, 19, 14, 1 hydrogen bonds and 211, 169, 183 non-bonding interactions respectively. The TRAF6 and TRIF interaction with CHIKV nsP2 showed various interactions between Asian, WA, and ECSA genotypes. TRAF6 showed 7, 4, 4 disulfide bonds, 17, 12, 14 hydrogen bonds and 160, 146, 186 non-bonding interactions, whereas nsP2-TRIF complex showed 0, 1, 1 disulfide bonds, 19, 16, 21 hydrogen bonds, and 179, 213, 230 non-bonding

interactions. The disulfide, hydrogen, and non-bonding interactions showed in red, blue, and orange colors (**Fig. S2 A-E**). The interactive residue involved in the disulfide and hydrogen bond formation was described in **Table S3-S7 and Fig. S3-S7**.

Discussion

The nsP2 is the largest protein among the nsPs of CHIKV. Based on the helicase and protease function, the nsP2 of CHIKV is targeted for molecular docking to achieve a significant result. Protein-Protein interaction at specific amino acid residues is considered essential in molecular docking. The bioinformatics approaches were considered a fast and reliable method to predict the potential interaction between virus-host cellular proteins, which played a significant role in developing antiviral drugs. The Three-dimensional (3D) structure of protein provides an opportunity to dock various immune cellular proteins against viral proteins [11,12]. Previously researchers conducted a study on docking-based interaction of proteins, an antimicrobial compound, and phytochemicals, and in vitro and in vivo experiments are still going on. Protein-protein docking has become an essential tool for antiviral drug discovery. Hence, the current study concentrated on the bioinformatics approach toward evaluating the antiviral effect of these six innate immune proteins on CHIKV nsP2. The CHIKV nsP2 was used as a target protein for these immune proteins to identify potential antiviral drugs for CHIKV infections [24].

The innate immune system provides the first line of defense in response to microbial infection. Pathogen-associated molecular patterns (PAMPs) are the
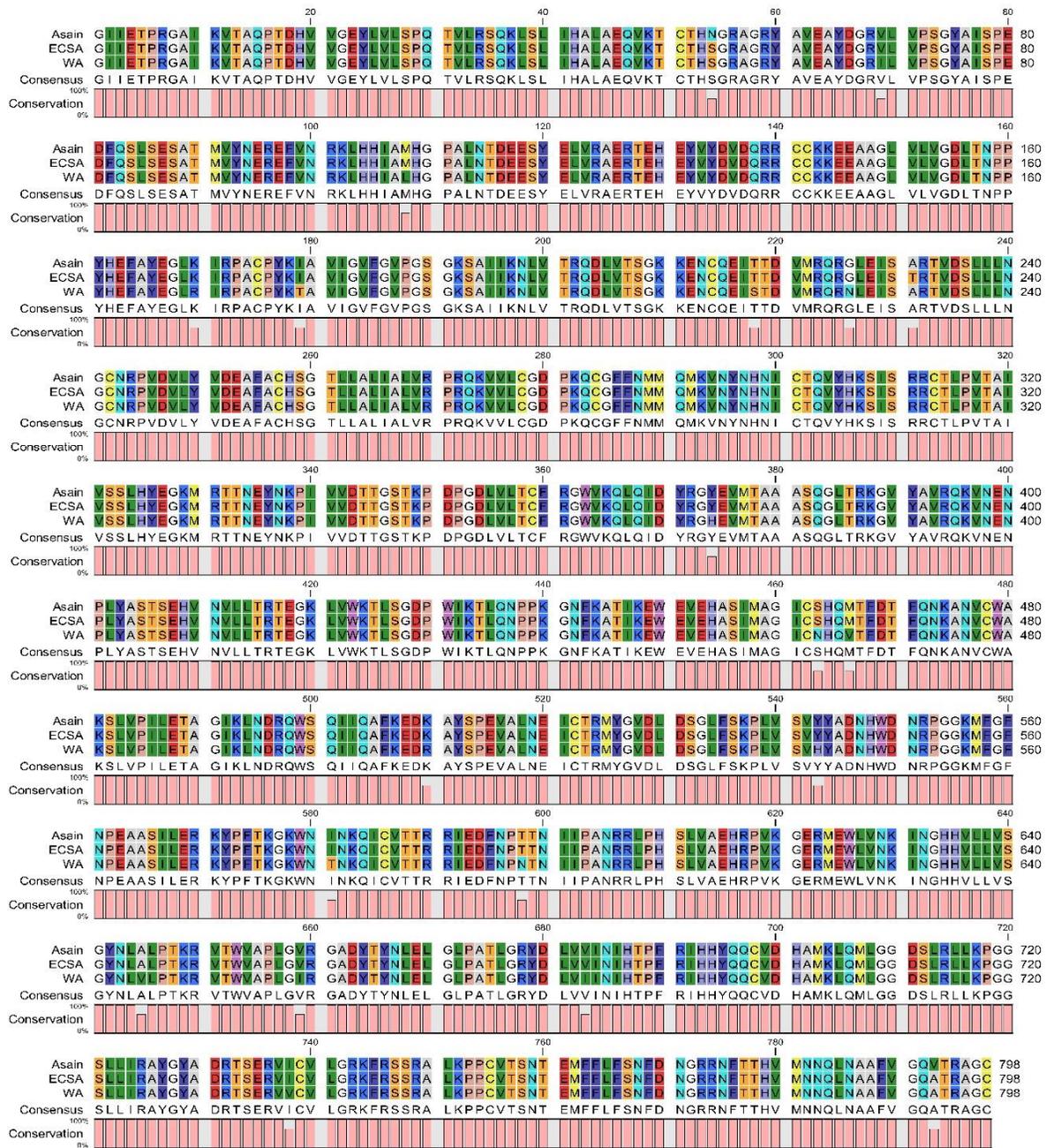
Fig 2: Consensus sequence of CHIKV nsP2 of Asian, WA, and ECSA genotypes. The consensus sequence has been shown by one-letter amino acid codes at the bottom above the bars. The bars show the degree of conserved amino acid at their respective position in terms of percentages.

the microbial substance that pathogen recognition receptors (PRRs) recognize on host cell surface receptors and activate the innate immune response. Thus, the innate immune response prevents or eliminates microbial infection. During viral infection, host PRRs recognize the viral substance through PAMPs and activate the interferon response to inhibit viral infection. Toll-like receptors and RIG-like

receptors are the two main types of PRRs; both recognize microbial infection and trigger the immune response [25,26].

RIG-I detects viral RNA in the cytoplasm, The conformational change in RIG-I or MDA-5 is required for the interaction between the tandem CARDs of the helicases with the CARD of IPS-1, which is incorporated into the mitochondrial outer membrane

by a transmembrane domain in its C-terminal site. Thus, this interaction induces interferon-sensitive genes and inflammatory inflammation. Type 1 interferon (IFNs) plays a significant role in the inhibition and clearance of viral infection [27]. *In vitro* study reported that RIG-I could detect synthesized RNA through 5' triphosphate of single-stranded RNA [28, 29]. In the current study, RIG-I was used as a positive control, RIG- I had strong bonding interaction with the PLP domain of CHIKV nsP2. The HADDOCK scores were relatively similar among the CHIKV genotypes; Asian, WA, and ECSA were followed (-103.7 ± 8.4, -105.7 ± 7.2, and -108.5 ± 2.3). The binding energies of the HADDOCK complex were shown in **Table 1**, and bonding interactions were shown in **Table S3 and Fig. S3**.

The conformational change in RIG-I or MDA-5 is required for the interaction between the tandem CARDs of the helicases with the CARD of IPS-1, which is incorporated into the mitochondrial outer membrane by a transmembrane domain in its C-terminal site [22]. While the interaction of these CARD–CARD domains, results in the propagation of downstream signaling leading to antiviral and inflammatory responses [30]. Previously, it has been reported that IPS-1 playing important role in antiviral immunity [31]. The bimolecular complex of CHIKV nsP2 and IPS-1 showed a higher HADDOCK score among the targeted host proteins. The HADDOCK of all three genotypes; Asian, WA, and ECSA were (-120.1 ± 4.4, -101.0 ± 6.1, and -114.3 ± 7.9). The binding energies of the HADDOCK complex were shown in **Table 1**, and bonding interactions were shown in **Table S4 and Fig. S4**.

TRAF3 plays an essential role in the promotion of signaling pathways by using interferon regulatory factors to produce IFNs which plays role in viral clearance [32]. TRAF3 regulates the stimulation of TLR-mediated mitogen-activated proteins like TLRs, Janus kinase, signal transducer, and activator of transcription pathways. These pathways are activated in response to viral infection; thus, the cell achieves the antiviral state. TRAF3 signal is essential for the activation of canonical and non-canonical nuclear factor-kappa B (NF-κB) pathways to activate antiviral immune response [33]. TRAF6 is one of the recently discovered mammalian proteins of the TRAFs family. TRAF6 regulates multiple signaling pathways, such as Proto-oncogene tyrosine-protein kinase (Src) family tyrosine kinase, an inhibitor of nuclear factor-κB, and mitogen-activated protein kinase. Myeloid lineage hematopoietic cells express receptors on the cell surface like receptor activators of nuclear factor

kappa beta, Cluster of differentiation 40, TLRs, and TRAF6, using this for the signaling to stimulate the innate and adaptive immunity towards pathogenic infection [34,35]. Both HADDOCK score and binding energies of TRAF3 and TRAF6 complexes were shown in **Table 1** and bonding interactions were shown in **Table S5-S6 and Fig. S5-S6**.

TRIF is a TIR-containing protein responsible for the activation of the TLR4-dependent pathway and Myeloid differentiation primary response 88 independent pathway, which activates NF-κB and IFN-β production. Interaction of TRIF on the C-terminal site with receptor-interacting protein 1 is essential for the activation of NF-κB. The TRIF signaling pathways regulates several transcription factors such as interferon regulatory factor 3, activator protein 1, and NF-κB, which leads to type 1 INF and cytokines production [36]. Both TRIF-dependent and independent pathways play a significant role in TLR signaling responses and in controlling viral replication and pathogenicity. We found a strong bonding affinity among the TRIF and nsP2 of CHIKV genotypes. The HADDOCK score and bonding interactions were shown in **Table S7 and Fig. S7**.

Conclusion

In conclusion, the binding affinity of innate immune proteins seems the most potent inhibitor against CHIKV nsP2. Both PLP and MTL domains of CHIKV nsP2 were involved in the interaction. Residues involved in interaction may be the potential target for the drugs and peptide inhibitors. The *in vivo* approach of the selected protein and interacting residues may be useful for designing pan-chikungunya antivirals in the future.

Authors' Contributions

HK designed the study, IA, SAK, S, and IN carried out *in silico* study. MS, LR, IK and NUQ helped in manuscript writing and revision.

Acknowledgments

Conflict of interest
The authors declare no conflict of interest.

References

[1] Bettadapura J, Herrero LJ, Taylor A, Mahalingam S. Approaches to the treatment of disease induced by chikungunya virus. Indian J Med Res. 2013;138:62-65.

[2] Stelitano D, Chianese A, Astorri R, Serretiello E, Zannella C, Folliero V, Galdiero M, Franci G, Crudele V, Vitiello M. Chikungunya virus: Update on molecular biology, epidemiology and current strategies. Translational Med Rep. 2019;3:1.

[3] Tsetsarkin KA, Chen R, Sherman MB, Weaver SC. Chikungunya virus: evolution and genetic determinants of emergence. Curr Opin Virology. 2011;4:310-7.

[4] Singh SK, Unni SK. Chikungunya virus: host-pathogen interaction. Reviews Med Viro. 2011; 2:78-88.

[5] Karpe YA, Aher PP, Lole KS. NTPase and 5′-RNA triphosphatase activities of Chikungunya virus nsP2 protein. PloS One. 2011;67:223-36.

[6] Das PK, Merits A, Lulla A. Functional cross-talk between distant domains of chikungunya virus non-structural protein 2 is decisive for its RNA-modulating activity. J Biol Chem. 2014;289:55-53.

[7] Fros JJ, Major LD, Scholte FE, Gardner J, van Hemert MJ, Suhrbier A, Pijlman GP. Chikungunya virus non-structural protein 2-mediated host shut-off disables the unfolded protein response. J Gen Virology. 2015;963:58-09.

[8] Das PK, Puusepp L, Varghese FS, Utt A, Ahola T, Kananovich DG, Lopp M, Merits A, Karelson M. Design and validation of novel chikungunya virus protease inhibitors. Antimicr agents Chem. 2016;6012:7382-95.

[9] Law YS, Wang S, Tan YB, Shih O, Utt A, Goh WY, Lian BJ, Chen MW, Jeng US, Merits A, Luo D. Interdomain flexibility of chikungunya virus nsP2 helicase-protease differentially influences viral RNA replication and infectivity. J Virology. 2021;956:01470-20.

[10] Law YS, Wang S, Tan YB, Shih O, Utt A, Goh WY, Lian BJ, Chen MW, Jeng US, Merits A, Luo D. Interdomain flexibility of chikungunya virus nsP2 helicase-protease differentially influences viral RNA replication and infectivity. J Virology. 2021;956:01470-20.

[11] Ngounou Wetie AG, Sokolowska I, Woods AG, Roy U, Deinhardt K, Darie CC. Protein–protein interactions: switch from classical methods to proteomics and bioinformatics-based approaches. Cell Molec Life Sci. 2014;712:205-28.

[12] Nourani E, Khunjush F, Durmuş S. Computational approaches for prediction of pathogen-host protein-protein interactions. Frontier Micro. 2015:94.

[13] Brito AF, Pinney JW. Protein–protein interactions in virus–host systems. Front Micro. 2017;8:15-57.

[14] Brito AF, Pinney JW. Protein–protein interactions in virus–host systems. Frontier Micro. 2017;8:1557.

[15] Franzosa EA, Xia Y. Structural principles within the human-virus protein-protein interaction network. Proc Natl Acad Sci U S A. 2011;108:538-543.

[16] Stringari LL, de Souza MN, de Medeiros Junior NF, Goulart JP, Giuberti C, Dietze R, Ribeiro-Rodrigues R. Covert cases of Severe Acute Respiratory Syndrome Coronavirus 2: An obscure but present danger in regions endemic for Dengue and Chikungunya viruses. PLoS One. 2021;16:0244-937.

[17] Simon F, Watson H, Meynard JB, de Santi VP, Tournier JN. What chikungunya teaches us about COVID-19. The Lancet Infectious Diseases. 2021;8:1070-1.

[18] Messaoudi A, Belguith H, Ben Hamida J. Homology modeling and virtual screening approaches to identify potent inhibitors of VEB-1 β-lactamase. Theoretical Biology and Medical Modelling. 2013 10(1):1-0.

[19] van Zundert GCP, Rodrigues JPGLM, Trellet M, et al. The HADDOCK2.2 Web Server: User-Friendly Integrative Modeling of Biomolecular Complexes. J Mol Biol. 2016;428:720-725.

[20] Lam Cabanillas, E., León Risco, A., León Risco, K., Llamo Hoyos, G., López Zavaleta, R., Luzuriaga Tirado, E., Mendoza Blas, A. and Huamán Saavedra, J. Molecular basis of COVID-19 pathogenesis and in silico studies of potential pharmacological treatment. Revista de la Facultad de Medi Huma. 2021;21:22.

[21] Jiménez-García B, Pons C, Fernández-Recio J. pyDockWEB: a web server for rigid-body protein–protein docking using electrostatics and desolvation scoring. Bioinformatics. 2013;29(13):1698-9.

[22] Saponaro A, Maione V, Bonvin AM, Cantini F. Understanding docking complexes of macromolecules using HADDOCK: the synergy between experimental data and computations. Bio-protocol. 2020 ;10-20.

[23] Nilofer C, Sukhwal A, Mohanapriya A, Kangueane P. Protein-protein interfaces are vdW dominant with selective H-bonds and (or) electrostatics towards broad functional specificity. Bioinform. 2017;13:164-173.

[24] Thompson MR, Kaminski JJ, Kurt-Jones EA, Fitzgerald KA. Pattern recognition receptors and the innate immune response to viral infection. Viruses. 2011;3:920-940.

[25] Kumar H, Kawai T, Akira S. Pathogen recognition by the innate immune system. Int Rev Immunol. 2011;30:16-34.

[26] Dixit E, Boulant S, Zhang Y, et al. Peroxisomes are signaling platforms for antiviral innate immunity. Cell. 2010;141:68-681.

[27] Su H, Liao Z, Yang C, Zhang Y, Su J. Grass Carp Reovirus VP56 Allies VP4, Recruits, Blocks, and Degrades RIG-I to More Effectively Attenuate IFN Responses and Facilitate Viral Evasion. Micro Spec. 2021;92:01000-21.

[28] Goubau D, Schlee M, Deddouche S, Pruijssers AJ, Zillinger T, Goldeck M, Schuberth C, Van der Veen AG, Fujimura T, Rehwinkel J, Iskarpatyoti JA. Antiviral immunity via RIG-I-mediated recognition of RNA bearing 5′-diphosphates. Nature. 2014;5147-522:372-5.

[29] Goubau D, Schlee M, Deddouche S, Pruijssers AJ, Zillinger T, Goldeck M, Schuberth C, Van der Veen AG, Fujimura T, Rehwinkel J, Iskarpatyoti JA. Antiviral immunity via RIG-I-mediated recognition of RNA bearing 5′-diphosphates. Nature. 2014;514:372-5.

[30] Yoneyama M, Onomoto K, Jogi M, Akaboshi T, Fujita T. Viral RNA detection by RIG-I-like receptors. Curr Opin Immunol. 2015;32:48-53.

[31] Häcker H, Tseng PH, Karin M. Expanding TRAF function: TRAF3 as a tri-faced immune regulator. Nat Rev Immunol. 2011;11:57-68.

[32] Nussinov R, Ma B, Tsai CJ. A broad view of scaffolding suggests that scaffolding proteins can actively control regulation and signaling of multienzyme complexes through allostery. Biochim Biophys Acta. 2013;183:20-29.

[33] Peng Y, Xu R, Zheng X. HSCARG negatively regulates the cellular antiviral RIG-I like receptor signaling pathway by inhibiting TRAF3 ubiquitination via recruiting OTUB1. PLoS Pathog. 2014;104:10040-41.

[34] Landström M. The TAK1–TRAF6 signalling pathway. Internat J Biochem Cell Biology. 2010;425:585-9.

[35] Landström M. The TAK1–TRAF6 signalling pathway. The Intern J Biochem & Cell Bio. 2010;425:585-9.

[36] Ullah MO, Sweet MJ, Mansell A, Kellie S, Kobe B. TRIF-dependent TLR signaling, its functions in host defense and inflammation, and its potential as a therapeutic target. J Leukoc Biol. 2016;10:22-31.