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Journal of Infection and Public Health 15 (2022) 781–787

Contents lists available at ScienceDirect

Journal of Infection and Public Health

journal homepage: www.elsevier.com/locate/jiph

Original article

Receptor binding domain of SARS-CoV-2 from Wuhan strain to Omicron B.1.1.529 attributes increased affinity to variable structures of human ACE2

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ARTICLE INFO

Article history:
Received 5 April 2022
Received in revised form 8 June 2022
Accepted 12 June 2022

Keywords:
SARS-CoV-2
Receptor binding domain
Angiotensin-converting enzyme
Omicron
Protein docking
Dynamic simulation

ABSTRACT

Background: COVID-19 is an infectious disease declared as a global pandemic caused by SARS-CoV-2 virus. Genomic changes in the receptor binding domain (RBD) region of SARS-CoV-2 led to an increased, infectivity in humans through interaction with the angiotensin-converting enzyme2 (ACE2) receptor. Simultaneously, the genetic variants in ACE2 provide an opportunity for SARS-CoV-2 infection and severity. We demonstrate the binding efficiencies of RBDs of SARS-CoV-2 strain with ACE2 variants of the human host.

Methodology: A Total of 615 SARS-CoV-2 genomes were retrieved from repository. Eighteen variations were identified contributing to structural changes in RBD that are distributed in 615 isolates. An analyses of 285 single nucleotide variances at the coding region of the ACE2 receptor showed 34 to be pathogenic. Homology models of 34 ACE2 and 18 RBD structures were constructed with 34 and 18 structural variants, respectively. Protein docking of 612 (34 *18) ACE2-RBD complexes showed variable affinities compared to wildtype Wuhan's and other SARS-CoV-2 RBDs, including Omicron B.1.1.529. Finally, molecular dynamic simulation was performed to determine the stability of the complexes.

Results: Among 612, the top 3 complexes showing least binding energy were selected. The ACE2 with rs961360700 variant showed the least binding energy (−895.2 Kcal/mol) on binding with the RBD of Phe160Ser variant compared to Wuhan's RBD complex. Interestingly, the binding energy of RBD of Omicron B.1.1.529 with ACE2 (rs961360700) structure showed least binding energy of −1010 Kcal/mol. Additionally, molecular dynamics showed structure stability for all the analysed complexes with the RMSD (0.22–0.26 nm), RMSF (0.11–0.13 nm), and Rg (2.53–2.56 nm).

Conclusion: In conclusion, our investigation highlights the clinical variants contributing to structural variants in ACE2 receptors that lead to efficient binding of SARS-CoV-2. Therefore, screening of these ACE2 polymorphisms will help detect COVID-19 risk population so as to provide additional care and for safe management.

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https://doi.org/10.1016/j.jiph.2022.06.004
1876-0341/

Introduction

The Interplay between pathogens and human hosts is a crucial process that causes infection. Susceptibility to disease depends on mutations that occur in disease and genetic polymorphisms in the human host. Genetic changes in the pathogen as well as in the host contribute to changes in the virulent character of the pathogen and immunological response in the host. Recent severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is one of the viral pathogens that adopted genetic changes from existing SARS family, causing lung infection and cytokine host response, leading to multi-organ failure [1]. The mode of viral transmission is well reported by Priyanka et al., 2020 [2]. Currently, SARS-CoV-2 has become a serious public health and economic crisis world-wide.

SARS-CoV-2 belongs to the genus of beta coronavirus like other SARS-CoV and Middle East respiratory syndrome coronavirus (MERS) [3] SARS-CoV-2 carries ~30 kb nucleotides as RNA genome that encodes structural proteins at 3′ ORFs and non-structural proteins (nsp) at 5′ end [4,5]. The structural proteins include spike (S), nucleocapsid (N), membrane (M), and envelope (E). Among them, envelope (E), membrane (M), and spike (S) proteins localized at viral surfaces are involved in host binding capacity, whereas the non-structural proteins are involved in replicase and transcriptase activities. According to world health organisation (WHO), early coronaviruses, SARS-CoV and MERS spread across 32 and 27 countries respectively, and were reported as global epidemic outbreak [6]. Whereas, the recent SARS-CoV-2 spread to most countries with prevalence of 89,707,115 cases and was described as a pandemic on 12th January 2020(www.covid19.who.int). Reports suggest SARS-CoV-2 shares 80 % homology with SARS-CoV and 50 % with MERS [7,8]. To date, several genomic variants have been reported in SARS-CoV-2 that may offer more virulence, presenting diverse clinical phenotypes among the affected population. Recently, Omicron B.1.1.529 was reported as a new variant of SARS-CoV-2 that emerged in South Africa in 2021 [9]. It spread rapidly among various other strains of SARS-CoV-2. Irrespective of any SARS-CoV-2 strain, the genetic variant or single nucleotide polymorphism (SNP) in a human host also makes them susceptible to SARS-CoV-2 in the attachment and progression of infection.

Amongst SARS-CoV-2 structural proteins, spike protein plays a vital role in attachment to the angiotensin converting enzyme 2 (ACE2) receptor of the human host, leading to infection. In recent times, the complex structure of spike protein with human ACE2 has been elucidated, showing the crucial residues involved in the communication. Particularly, the receptor binding domain (RBD) of spike protein containing 253 aminoacids structurally interacts with the host ACE2 receptor that initiates the entry of viral particle into the human host. Growing evidence on genetic changes in RBD region of SARS-CoV-2 and the SNP in ACE2 receptor of host posses various dynamics of interaction [10]. Understanding the dynamic interaction between the viral spike and host ACE2 based on genetic variations will suggest crucial ACE2 polymorphism, causing susceptibility to the host in the attachment of SARS-CoV-2.

In this study, we investigated the molecular interaction between the RBD of spike and human ACE2 receptor based on protein-protein docking method by implementing previously observed variants in both the RBD of spike and human ACE2 receptor. Our approach (Fig. 1) uses high throughput genomic sequence data of SARS-CoV-2 and human ACE2 sequences from repositories, that identifies, (i) the genetic variants localized in RBD that contributed to structural changes in the spike protein of SARS-CoV-2 isolated from Indian patients. (ii) thirty four pathogenic single nucleotide variant causing structural changes in human ACE2 protein. (iii) Protein interactions between human ACE2 receptor with SARS-CoV-2 RBD based on the amino acid variations were derived using protein modeling and molecular docking. Additionally, total binding energies were assessed for the selected complexes based on MM-GBSA which confirmed significant interaction between ACE2 receptor and SARS-CoV-2 RBD. Overall, our analysis provides the crucial RBD variants in SARS-CoV-2 and significant polymorphism in human ACE2 receptor causing increased susceptibility to infection.

Methodology

RBD sequence and phylogenetic analysis of SARS-CoV-2 Indian isolates

The genomic and proteomic sequences of SARS-CoV-2 isolated from Indian participants were retrieved from the Global Initiative on Sharing All Influenza Data(GISAID) repositories (https://www.gisaid.org/). From each of the collected sequence, the proteomic region with 253 amino acids contributing to RBD was extracted,. The collected RBD sequences were subjected to multiple sequence alignment using MUSCLE tool from EBI database (www.ebi.ac.uk/Tools/msa/muscle) to record the variants between sequences based on position and amino acid changes. Additionally, the phylogenetic tree was generated using MEGA version 10 software [11]. We adopted the maximum likelihood method with 1000 bootstrap values for distance calculation between the SARS-CoV-2 isolates from various regions of India based on the RBD sequences.

ACE2 variants and pathogenic prediction

The human gene as well as the protein sequence of the ACE2 receptor were collected from the UniProt website (www.uniprot.org) using accession ID: Q9BYF1. The presence of genetic variants localised in ACE2 receptor was searched from? NCBI, SNP database (https://www.ncbi.nlm.nih.gov/snp/). Appropriate filters were set to extract the single nucleotide variance (SNV) only at the coding region of ACE2 gene, influencing the amino acid variation. Further, the pathogenic properties of ACE2 protein influenced by the amino acid variation appropriate to the SNV was determined using Ensembl Variation - Pathogenicity predictions tool that includes ??? such as (1) combined annotation dependent depletion (CADD), (2) PolyPhen-2, (3) sorting intolerant from tolerant (SIFT), and (4) rare exome variant ensemble learner (REVEL) (accessed on November 2021). Among 285 variants analysed, 34 pathogenic variants were identified, and each variant was introduced into the ACE2 protein (wild-type: Q9BYF1) sequence to search for the variant structure using PDB-BLAST (https://blast.ncbi.nlm.nih.gov/Blast.cgi) (accessed on November 2021).

Molecular modelling

Due to the lack of protein structure in all the 34 ACE2 variants, the homology modelling was conducted to generate protein structures using Swiss-model (https://swissmodel.expasy.org/). A similar attempt was made to generate a structure for the RBD of SARS-CoV-2. Template structure was searched in protein data bank (PDB: https://www.rcsb.org/), which showed (*PDB ID:1R42*)a native human angiotensin converting enzyme-related carboxypeptidase as a template for human ACE2 structure (accessed on November 2020). Hence, PDB ID: 1R42 was used to generate protein structures with 34 ACE2 variants. Similarly, the variant structures of RBD were generated from the template structure *PDB ID:7A92*, a spike glycoprotein. Additionally, the RBD structure for the SARS-CoV-2 Omicron variant (B.1.1.529) was generated from the protein sequence retrieved from NCBI GenBank: (Accession no. UIN78098.1). Further, the constructed (ACE2 and RBD) structures were individually optimized based on discrete optimized protein energy (DOPE) [12] and verified using PROCHECK (www.ebi.ac.uk/thornton-srv/software/PROCHECK/) and VERIFY-3D (www.doe-mbi.ucla.edu/Services/Verify_3D) tools.

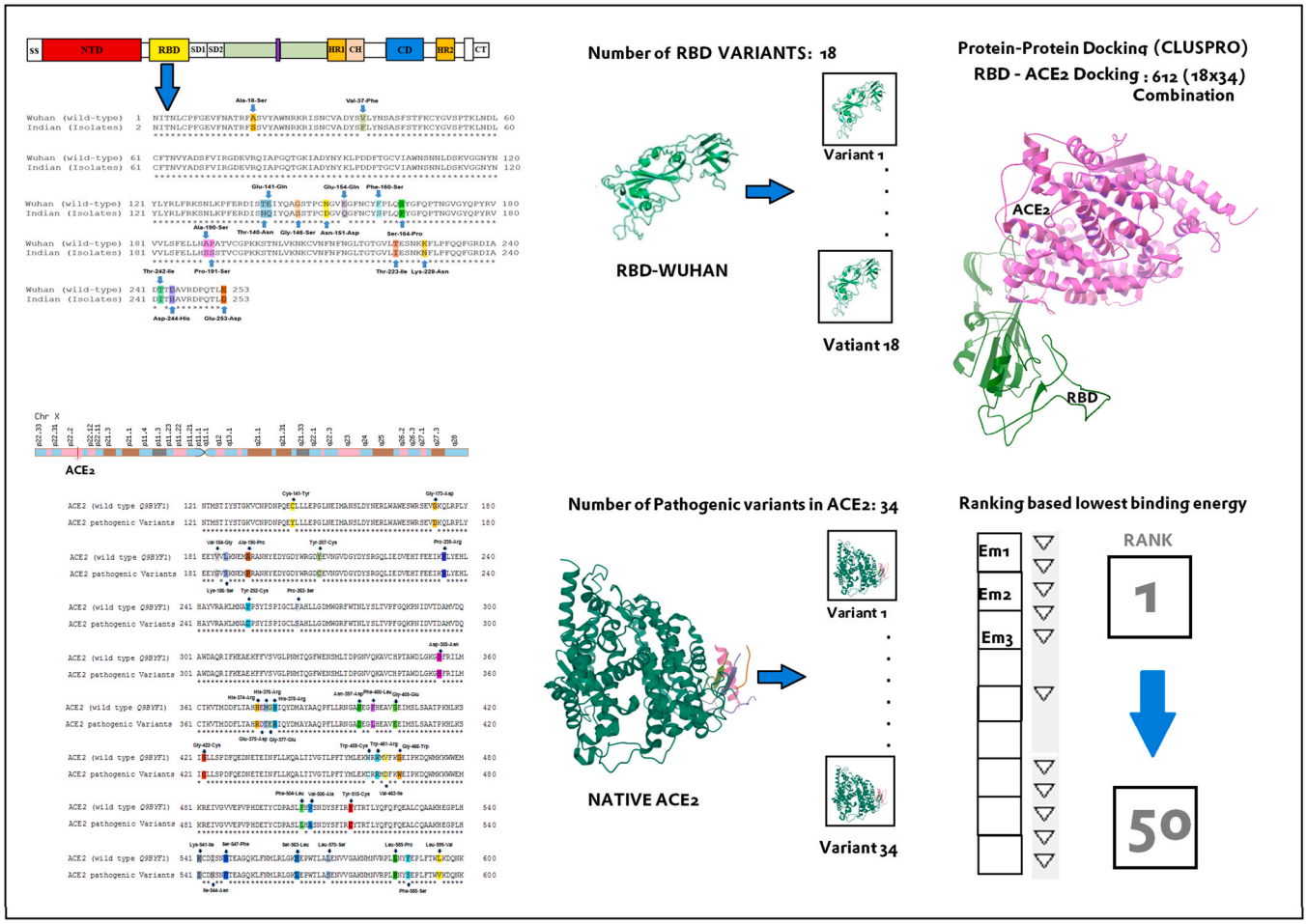

Fig. 1. Workflow of study design. Our approach uses high throughput genomic sequence data of SARS-CoV-2 and human ACE2 sequence from the repositories, that identifies (A) Eighteen amino acid variants.
localized in RBD contributing structural changes in the spike protein of SARS-CoV-2 isolated from Indian patients. (B) thirty-four pathogenic single nucleotide variants causing structural changes in human ACE2 protein. (C) Structures of RBD with18 variants generated using modeling tools. (D) Structures of ACE2 receptor proteins with 34 variants using modeling tools. (E) 612 combinations of protein interactions between human ACE2 with SARS-CoV-2 RBD were established using molecular protein docking. (F) Ranking and selecting the top 3 ACE2-RBD complexes based on the lowest energy.

Protein-protein docking

CLUS PRO 2.0 (www.cluspro.org/) server was used to determine the protein-protein docking between the 34 variants of ACE2 receptor and the 18 variants of RBD. Almost, 612 (34*18) docking prediction jobs were submitted to the server for each ACE2-RBD complex. Three interacting complexes were selected based on binding energy. The least binding energy of the top 3 complexes was compared with the binding energy derived between wild-type structure of ACE2 and RBD isolates of Wuhan. Additionally, the ACE2 protein of selected top3 was subjected to protein-protein docking with the RBD of Omicron variant (B.1.1.529) to determine the change in affinity due to the evolution of SARS-CoV-2.

Molecular dynamics simulation

Based on the ranked lowest energies, three top ranked complexes were selected TOP1: ACE2 (rs961360700) and RBD (F490S); TOP2: ACE2 (rs1305384714) and RBD (F490S); and TOP3: ACE2 (rs148036434) and RBD (F490S)). In addition to these, the native complex (wildtype ACE2-Wuhan RBD), the omicron complexes: ACE2 (rs961360700) and RBD (Omicron); ACE2 (rs1305384714) and RBD (Omicron), ACE2(rs148036434) and RBD (Omicron) were subjected to molecular dynamic study using GROMACS 2020 version. The OPLS-AA (Optimize Potential for Liquid systems-all atom) force field was applied and solvated using SPC water in a cubic box with the protein complex. Furthermore, the system was neutralized, energy-minimized,and equilibration(NVT and NPT) was performed. Finally, molecular simulation was carried out for 100nanosecond(ns) and the structural stability was assessed based on root mean square deviation (RMSD), root mean square fluctuation (RMSF), and radius of gyration (Rg).

Results

RBD sequence and phylogenetic analysis of SARS-CoV-2 Indian isolates

The genomic sequences of SARS-CoV-2 isolated from the COVID-19 infected Indian patients were collected from the GISAID repository. As on July 15th,2020, 615 genomes were retrieved covering 19 states of India, such as Andhra Pradesh, Assam, Bihar, Delhi, Gujarat, Haryana, Karnataka, Kerala, Ladakh, Madhya Pradesh, Maharashtra, Odisha, Punjab, Rajasthan, Tamil Nadu, Telangana, Uttar Pradesh, West Bengal, Jammu and Kashmir. Along with the genome and spike protein sequences, information about the data accession identifier, submitted Lab, date of sampling, sample contributor's geographical region in India, gender, age, and health status of the sample contributor were recorded (Supplementary Table.1) From each spike protein sequence, the region contributing to RBD was dissected. Multiple sequence alignment of 615 RBD sequences

showed presence of 18 variants in RBD that are distributed throughout India. Among the 18 RBD variants, one was noticed to be identical with the SARS-CoV-2 RBD of Chinese Wuhan population that spread across all the 19 analysed states of India, suggesting the transmission of COVID-19 between these regions. Despite variations in the RBD domain, most studies report the interaction between SARS-CoV-2 RBD and the human ACE2 receptor as an entry point for infection.

ACE2 variants and pathogenic prediction

Human ACE2 receptor gene and protein sequence were retrieved from the UniProt website using accession ID: Q9BYF1 containing 804 aminoacids contributing to the M2 peptidase and collectrin domain. Further, the analysis of ACE2 gene sequence using SNP database showed the presence of 285 single nucleotide variations (SNVs) in the coding region that may contribute to the variation in structures. Among the 285 SNVs, 34 were predicted to have adverse affects rendered by amino-acid variations in ACE2 based on CADD, PolyPhen-2, SIFT, and REVEL (Table 1). Each variant was introduced into the ACE2 protein sequence to search for its structure using protein PDB-BLAST. Currently, no resolved structure is available in the PDB database for the variant induced ACE2s. Hence,we use homology modelling to construct structures for all the 34 variants of ACE2. Similarly, 18 RBD structures were generated, with each variant having been noticed in the RBD of SARS-CoV-2 isolated from the Indian population. In addition to these 18, the RBD structure of the Omicron variant (B.1.1.529) was constructed for later comparison. All the generated structures were DOPE optimized and verified using PROCHECK and VERIFY-3D tools.

ACE2-RBD docking

Protein-protein docking was conducted using the CLUS PRO 2.0 (https://cluspro.org/) server between the 34 variants of the ACE2 receptor and 18 variants of RBD. The predicted interactions between 612 (34*18) ACE2-RBD complexes showed efficient interactions based on the lowest energy ranging from-895.2 Kcal/mol to −733.6 Kcal/mol (Supplementary Table. 2). Among the 612 combinations, the top 50 were assessed based on lowest energy (−895.2 to −869.7 Kcal/mol) (Fig. 2). The interaction of human ACE2 with rs961360700(D355N) and RBD with F490Svariant showed least energy of −895.2 Kcal/mol that most likely had efficient binding with thehuman host. Briefly, the hydrogen bond interactions between ACE2 rs961360700 variant and RBD variant of SARS-CoV-2 were found at TYR255, PHE603, ASN159, GLU483, GLU160, ASP136, PRO612, PRO135, ASP157, ALA251, LEU156, and TRP606 of ACE2 with PHE235, ASP98, GLN233, LYS132, LYS227, LYS228, HIS189, and ARG237of RBD, respectively. Similarly, the molecular interactions were assessed for other top ranked ACE2-RBD complexes, which showed several conserved interactions irrespective of SNVs (Fig. 2). Alternately, few specific interactions were also noticed between ACE2-RBD variants, suggesting the influence of SNVs (Fig. 2). Top 50 binding affinities were compared with the wild-type (complex A: Wuhan SARS-CoV-2 isolates) and the RBDs of other Indian variants (Supplementary Table 2). Based on the lowest ranked energies, three top ranked complexes were selected, complex B: ACE2 (rs961360700) and RBD (F490S); complex C: ACE2 (rs1305384714) and RBD (F490S), complex D: ACE2 (rs148036434) and RBD (F490S). Simultaneously, the ACE2 structures of top 3 variants were docked with the RBD of Omicron (B.1.1.529) and showed binding energies of −1010.3, −1024.1, and −923.8 Kcal/mol for the complexes E: ACE2 (rs961360700) and RBD (Omicron); complex F: ACE2 (rs1305384714) and RBD (Omicron), complex G: ACE2(rs148036434) and RBD (Omicron), respectively.

Table 1
Predicated pathogenic variants in ACE2 receptor.

rs-id	Allele	Amino acid variation	SIFT	Poyphen	CADD	REVEL
rs1391451327	C/T	Cys141Tyr	D	PD	LB	LDC
rs865852627	C/T	Gly173Ser	D	PD	LB	LB
rs758142853	A/C/**G**	Val184Gly	D	PD	LB	LDC
rs750052167	A/G	Leu186Ser	D	PD	LB	LDC
rs765733397	C/G	Ala191Pro	D	PD	LB	LDC
rs754237613	T/C	Tyr207Cys	D	PD	LB	LDC
rs1172580854	G/C	Pro235Arg	D	PD	LB	LDC
rs771769548	T/C	Tyr252Cys	D	PD	LB	LDC
rs200745906	G/A	Pro263Ser	D	PD	LB	LDC
rs961360700	C/T	Asp355Asn	D	PD	LB	LB
rs1309363592	T/C	His374Arg	D	PD	LB	LDC
rs1395782023	C/A	Glu375Asp	D	PD	LB	LB
rs754076967	A/G	Met376Thr	D	PD	LB	LDC
rs767462182	C/T	Gly377Glu	D	PD	LB	LDC
rs142984500	T/C	His378Arg	D	PD	LB	LDC
rs1365935088	T/C	Asn397Asp	D	PD	LB	LDC
rs1214851578	A/G	Phe400Leu	D	PD	LB	LDC
rs1418150776	C/T	Gly405Glu	D	PD	LB	LDC
rs761489852	C/A	Gly422Cys	D	PD	LB	LB
rs11798104	C/A	Trp459Cys	D	PD	LB	LDC
rs1476137643	A/G	Trp461Arg	D	PD	LB	LDC
rs1169303958	A/T	Val463Ile	D	PD	LB	LDC
rs774978137	C/A	Gly466Trp	D	PD	LB	LDC
rs1285805675	G/T	Phe504Leu	D	PBD	LB	LB
rs775181355	A/G	Val506Ala	D	PD	LB	LDC
rs1263424292	T/C	Yr515Cys	D	PD	LB	LDC
rs889263894	T/A	Lys541Ile	D	PD	LB	LB
rs769821600	A/T	Ile544Asn	D	PD	LB	LDC
rs373025684	G/A/C	Ser547Cys	D	PD	LB	LDC
rs375352455	G/A	Ser563Leu	D	PD	LB	LB
rs1305384714	A/G	Leu570Ser	D	PD	LB	LDC
rs1173089368	A/G	Leu585Pro	D	PD	LB	LDC
rs760929786	A/G	Phe588Ser	D	PD	LB	LDC
rs148036434	G/C	Leu595Val	D	PD	LB	LDC

Thirty-four variations were predicted to have adverse affect rendered by amino-acid variations in ACE2 based on CADD, PolyPhen-2, SIFT, and REVEL.
D- Deleterious; PD - Probably damaging; PBD Possibly damaging; LB - likely Benign; B - Benign; LDC - Likely Disease Causing.
Supplementary Table 1: Information demonstrating the data used in this study
Information such as accession number, submitted Lab, date of sampling, sample contributor geographical location, gender, age and health Status of the sample contributor were recorded.
Supplementary Table. 2: Ranking the interaction based on lowest energy
The predicted interaction between 612 (34*18) ACE2-RBD complex showing efficient interaction based on the lowest energy ranging from −895.2 Kcal/mol to −733.6 Kcal/mol.

Molecular dynamics simulation

Seven individual molecular dynamic simulations were performed: complex A: ACE2 wild-type and RBD (Wuhan strain); complex B: ACE2 (rs961360700) and RBD (F490S); complex C: ACE2 (rs1305384714) and RBD (F490S), complex D: ACE2 (rs148036434) and RBD (F490S), complex E: ACE2 (rs961360700) and RBD (Omicron); complex F:ACE2 (rs1305384714) and RBD (Omicron), complex G: ACE2(rs148036434) and RBD (Omicron). Based on the RMSD and RMSF, the structural stability of ACE2-RBD variant systems was evaluated. The average RMSD values for all the complexes A-G were 0.22, 0.26, 0.23, 0.24, 0.24, 0.25, and 0.24 nm, respectively (Fig. 3A). No significant deviation was noticed in RMSD plots for each complex from A to G, which suggests that the complexes were highly stable throughout 100 ns of simulation. Similarly, the RMSF was calculated to determine the flexibility based on the atoms of the complex. All the seven systems demonstrated almost a similar pattern of fluctuation during simulation. The RMSF average for complexes from A-G were 0.11, 0.13, 0.11, 0.12, 0.11, 0.12, and 0.12 nm, respectively which reveals stability of the ACE2 on RBD binding (Fig. 3B). Likewise, the average radius of gyration (Rg) values calculated for the complexes A-G were, 2.53, 2.54, 2.54, 2.55, 2.56, 2.53,

RBD	ARG237	ARG237	ARG237	ASP98	GLN233	HIS189	HIS189	LYS132	LYS132	LYS227	LYS228	LYS228	PHE235	GLN234	LYS132	LYS227	SER190	LYS227	ARG237	Lowest Energy (Kcal/mol)
Interaction																				
ACE2	ALA251	ASP157	LEU156	PHE603	ASN159	PRO612	TYR255	GLU483	TRP606	GLU160	ASP136	PRO135	TYR255	TYR255	THR608	ASP157	TYR613	ASN134	PRO253	
Rank_1	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	X	X	X	X	X	X	-895.2
Rank_2	✓	✓	✓	✓	X	✓	✓	✓	✓	✓	✓	✓	✓	X	X	X	X	X	X	-894.9
Rank_3	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	X	X	X	X	X	X	-894.8
Rank_4	✓	✓	✓	✓	X	✓	✓	✓	✓	✓	✓	✓	✓	X	X	X	X	X	X	-894.6
Rank_5	✓	✓	✓	✓	X	✓	✓	✓	✓	✓	✓	✓	✓	X	X	X	X	X	X	-894.2
Rank_6	✓	✓	✓	✓	X	✓	✓	✓	✓	✓	✓	✓	✓	X	X	X	X	X	X	-894
Rank_7	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	X	X	X	X	X	X	-893.9
Rank_8	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	X	X	X	X	X	X	-893.9
Rank_9	✓	✓	✓	✓	X	✓	✓	✓	✓	✓	✓	✓	✓	X	X	X	X	X	X	-893.9
Rank_10	✓	✓	✓	✓	X	✓	✓	✓	✓	✓	✓	✓	✓	X	X	X	X	X	X	-893.9
Rank_11	✓	✓	✓	✓	X	✓	✓	✓	✓	✓	✓	✓	✓	X	X	X	X	X	X	-893.9
Rank_12	✓	✓	✓	✓	X	✓	✓	✓	✓	✓	✓	✓	✓	X	X	X	X	X	X	-893.9
Rank_13	✓	✓	✓	✓	X	✓	✓	✓	✓	✓	✓	✓	✓	X	X	X	X	X	X	-893.9
Rank_14	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	X	X	X	X	X	X	-893.9
Rank_15	✓	✓	✓	✓	X	✓	✓	✓	✓	✓	✓	✓	✓	X	X	X	X	X	X	-893.9
Rank_16	✓	✓	✓	✓	X	✓	✓	✓	✓	✓	✓	✓	✓	X	X	X	X	X	X	-893.9
Rank_17	✓	✓	✓	✓	X	✓	✓	✓	✓	✓	✓	✓	✓	X	X	X	X	X	X	-893.1
Rank_18	✓	✓	✓	✓	X	✓	✓	✓	✓	✓	✓	✓	✓	X	X	X	X	X	X	-893.1
Rank_19	✓	✓	✓	✓	X	✓	✓	✓	✓	✓	✓	✓	✓	X	X	X	X	X	X	-892.6
Rank_20	✓	✓	✓	✓	X	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	X	X	X	X	-891.2
Rank_21	✓	✓	✓	✓	X	✓	✓	✓	✓	✓	✓	✓	✓	✓	X	X	X	X	X	-882.9
Rank_22	✓	✓	✓	✓	X	✓	✓	✓	✓	✓	✓	✓	✓	X	X	X	X	X	X	-882.4
Rank_23	✓	✓	✓	✓	X	✓	✓	✓	✓	✓	✓	✓	✓	X	X	X	X	X	X	-882.3
Rank_24	✓	✓	✓	✓	X	✓	✓	✓	✓	✓	✓	✓	✓	X	X	X	X	X	X	-882.2
Rank_25	✓	✓	✓	✓	X	✓	✓	✓	✓	✓	✓	✓	✓	✓	X	X	X	X	X	-881.7
Rank_26	✓	✓	✓	✓	X	✓	✓	✓	✓	✓	✓	✓	✓	X	X	X	X	X	X	-881.5
Rank_27	✓	✓	✓	✓	X	✓	✓	✓	✓	✓	✓	✓	✓	✓	X	X	X	X	X	-881.5
Rank_28	✓	✓	✓	✓	X	✓	✓	✓	✓	✓	✓	✓	✓	✓	X	X	X	X	X	-881.5
Rank_29	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	X	X	X	X	X	X	-881.5
Rank_30	✓	✓	✓	✓	X	✓	✓	✓	✓	✓	✓	✓	✓	X	X	X	X	X	X	-881.5
Rank_31	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	X	X	X	X	X	X	-881.4
Rank_32	✓	✓	✓	✓	X	✓	✓	✓	✓	✓	✓	✓	✓	X	X	X	X	X	X	-881.4
Rank_33	✓	✓	✓	✓	X	✓	✓	✓	✓	✓	✓	✓	✓	X	X	X	X	X	X	-881.4
Rank_34	✓	✓	✓	✓	X	✓	✓	✓	✓	✓	✓	✓	✓	X	X	X	X	X	X	-881.4
Rank_35	✓	✓	✓	✓	X	✓	✓	✓	✓	✓	✓	✓	✓	X	X	X	X	X	X	-880.7
Rank_36	✓	✓	✓	✓	X	✓	✓	✓	✓	✓	✓	✓	✓	X	X	X	X	X	X	-880.6
Rank_37	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	X	X	X	X	X	X	-880.1
Rank_38	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	X	X	X	X	X	X	-879.7
Rank_39	✓	✓	✓	✓	●	✓	✓	✓	✓	✓	✓	✓	✓	✓	X	X	X	X	X	-878.6
Rank_40	✓	✓	✓	✓	X	✓	✓	✓	✓	✓	✓	✓	✓	✓	X	✓	X	X	X	-878.2
Rank_41	✓	✓	✓	✓	X	✓	✓	✓	✓	✓	✓	✓	✓	✓	X	X	X	X	X	-878
Rank_42	✓	✓	✓	✓	X	✓	✓	✓	✓	✓	✓	✓	✓	✓	X	X	X	X	X	-878
Rank_43	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	X	X	X	X	X	X	-876.7
Rank_44	✓	X	✓	✓	✓	✓	✓	✓	✓	X	✓	✓	✓	X	X	✓	X	X	X	-875.6
Rank_45	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	X	X	X	X	X	X	-875.6
Rank_46	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	X	X	X	✓	X	X	-874.5
Rank_47	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	X	X	X	X	X	X	-872.8
Rank_48	●	✓	✓	✓	✓	✓	✓	✓	✓	X	✓	✓	✓	X	X	X	X	✓	✓	-872.3
Rank_49	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	X	X	X	X	X	X	-869.8
Rank_50	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	X	X	X	X	X	X	-869.7

Fig. 2. Molecular interaction between ACE2-RBD complexes. The top ranked ACE2-RBD complexes showing that amino acids contributing to the interaction. Checkmark represents the presence of interaction, and the Cross mark represents the absence of interaction. Several conserved interactions were observed irrespective of variations.

and 2.55 nm, respectively (Fig. 3C), which demonstrated the compactness of protein complex.

Discussion

Evolution and dynamic changes in the SARS-CoV genome caused a wide spread of infection throughout the globe. WHO report suggested high mortality due to SARS-CoV-2 compared to other previously known SARS-CoV and MERS [13]. Studies suggest that genetic variants in the viral spike protein encoding gene of SARS-CoV-2 gain the ability of cross transmission [14,15]. Particularly, the recent SARS-Co-2 was considered to have come from the Rhinolophus affins bat that was transferred to human through an intermediate animal host, pangolin [16]. Also, these genetic changes contribute to the effective binding of SARS-CoV-2 to the human host ACE2 receptor to cause infection. Most coronavirus strains, including SARS-CoV, NL63, and SARS-CoV-2 use the human ACE2 receptor as an entry point for infection [17]. The ACE2 gene encodes a metalloprotease, expressed widely in the epithelial cells of most organs, including the lungs. ACE2 has been linked to vasodilatory and antifibrotic effects [18]. Changes in ACE2 expression have been linked to hypertension, diabetes, and cardiovascular disease [18]. Similarly, genetic polymorphisms in ACE2 gene cause susceptibility to hypertension in African-American, Brazilian, Chinese, Canadian, and Indian populations [19–23]. However, it remains unknown how genetic polymorphisms in human ACE2 influence the susceptibility to SARS-CoV-2. In this study, we investigated the molecular interactions between twelve RBD variants of spike protein of Indian SARS-CoV-2 isolates with thirty-four pathogenic variants of human ACE2 receptor based on the protein docking method. Overall, 408 combinations of protein interactions were assessed that showed an increased affinity between human ACE2 receptor and RBD, causing susceptibility to infection compared to RBD variants of Wuhan SARS-CoV-2 isolates.

Among 615 SARS-CoV-2 Indian isolates, 18 variants were noticed, contributing to the structural changes in the RBD region. Also, these variants were widely spread throughout 19 Indian states. For instance, the variant Ala18Ser of RBD that can be compared to the Wuhan SARS-CoV-2 isolates was distributed in Telangana and Odisha in India. Similarly,one of the Indian SARS-CoV-2 isolate showed identical RBD with Wuhan SARS-CoV-2 was seen to be distributed across Andhra Pradesh, Assam, Bihar, Delhi, Gujarat, Haryana, Karnataka, Kerala, Ladakh, Madhya Pradesh, Maharashtra, Odisha, Punjab, Rajasthan, Tamil Nadu, Telangana, Uttar Pradesh, West Bengal, Jammu and Kashmir. The presence of identical RBDs across the Indian regions suggest possible human transmission of SARS-CoV-2 between the analysed regions. Also, the coding variants of human ACE2 receptors of various ethnicities were extracted from public resources due to less availability of variants reported specific to the Indian population. Of human ACE2 receptor variants, thirty-four noticed pathogenicity based on the prediction methods. Comparative molecular modelling revealed fifty ACE2 variations contributing to high inter-molecular affinity between SARS-CoV-2 RBD variants. Interestingly, the ACE2 variants rs961360700 (D355N), rs1305384714 (L570S), rs148036434 (L595V), rs774978137(G466W), and rs1365935088 (N397D) with RBD variants showed efficient binding energy between −895.2 and-894.2 Kcal/mol (Fig. 2). Additionally, binding energy was compared to Wuhan isolates complex and showed binding energy ranging between −878 and-804.1 Kcal/

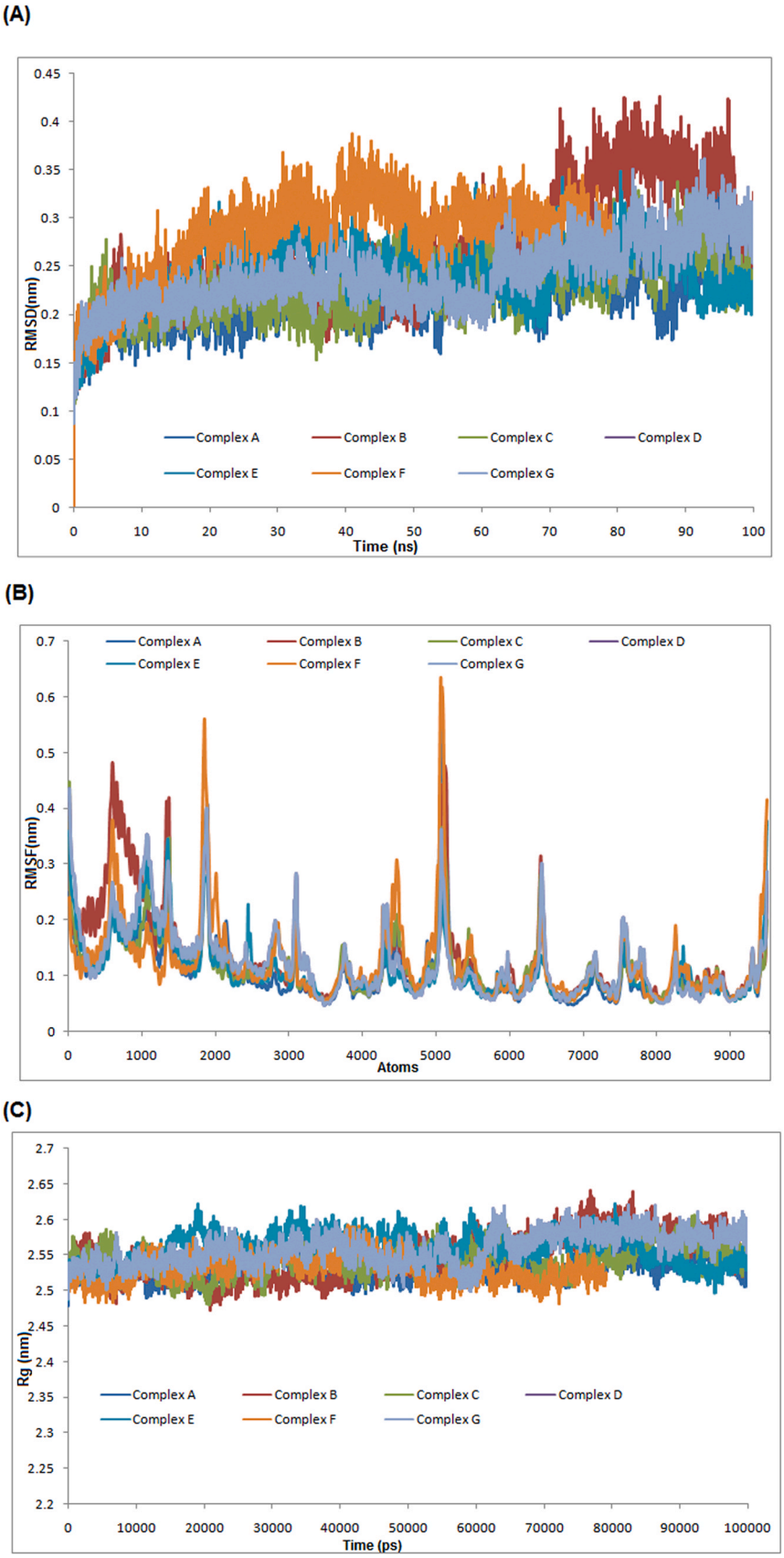

Fig. 3. Molecular dynamic simulation. Molecular dynamics of ACE2-RBD generated over 100 ns and the outcome reported based on RMSD, RMSF and Rg (A) RMSD versus time graph (ns) (B) RMSF trajectories based on atoms of the complexes, and (c) Rg versus time graph of the complexes.

mol. However, the noticed RBD variants of Indian SARS-CoV-2 genomes were particularly from Maharashtra (F160S) and had the potential to contribute to increased susceptibility, if the individual attributed to any one of the following ACE2 variants rs961360700 (D355N), rs1305384714 (L570S), and rs148036434 (L595V). Although, there are several significant binding efficient complexes, we tried to show the importance of those with ACE2-RBD protein complex to have a significantly higher affinity than the Wuhan SARS-CoV-2 RBD region one (so called native) (Supplementary Table 2). On the other hand, the influence of recently emerging Omicron (B.1.1.529) assessed based on the interaction of RBD with the selected ACE2 receptor variants showed high affinity and retained its stability till 100 ns of molecular dynamic simulation.Omicron sparked worldwide and has now spread to almost 75 countries, resulting in numerous speculations concerning its origin and degree of infectivity [24]. The results obtained from our docking analysis provide a molecular basis for enhanced infectivity of the omicron variant. Recently, Lupala et al. executed protein docking between ACE2 and omicron but did not compare the binding affinity with the other COVID strains [25]. Kumar et al. [26], on the other hand, compare how well delta and omicron bind to the ACE2 receptor. However, the dynamic information of ACE2 variants and the RBD interaction has not been illustrated. Our study has an advantage in three folds. First, we described omicron RBD's binding efficacy with the screened ACE2 clinical variants. Secondly, we examined the most frequently reported COVID isolates. Third, we use molecular dynamic simulation to figure out how a COVID genetic variation affects ACE2 clinical variations.

Conclusion

In conclusion, our investigation highlights the clinical variants of ACE2 that lead to efficient binding of SARS-CoV-2 isolated from the Indian population. Our study identifies candidate polymorphisms in both human ACE2 and RBD of SARS-CoV-2 showing more efficient binding than Wuhan SARS-CoV-2 isolates. In particular, the existence of ACE2 variants rs961360700 (D355N), rs1305384714 (L570S) and rs148036434 (L595V) is an important candidate offering efficient binding of SARS-CoV-2, including the Evolution and dynamic changes in the SARS-CoV-2 variant. Therefore, investigating and screening of these polymorphisms will help in detecting COVID19 susceptible populations to provide additional care and safe management.

Competing interests

None declared

Acknowledgments

This work was supported by the Taif University Researchers Supporting Program (Project number: TURSP-2020/238), Taif University, Saudi Arabia

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jiph.2022.06.004.

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