Original Research Article

Impact of B.1.617 and RBD SARS-CoV-2 variants on vaccine efficacy: An *in-silico* approach

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A B S T R A C T

Purpose: The existing panels of COVID-19 vaccines are based on the spike protein of an earlier SARS-CoV-2 strain that emerged in Wuhan, China. However, the evolving nature of SARS-CoV-2 has resulted in the emergence of new variants, thereby posing a greater challenge in the management of the disease. India faced a deadlier second wave of infections very recently, and genomic surveillance revealed that the B.1.617 variant and its sublineages are responsible for the majority of the cases. Hence, it's crucial to determine if the current vaccines available can be effective against these variants.
Methods: To address this, we performed molecular dynamics (MD) simulation on B.1.617 along with K417G variants and other RBD variants. We studied structural alteration of the spike protein and factors affecting antibody neutralization and immune escape *via In silico* docking.
Results: We found that in seven of the 12 variants studied, there was a structural alteration in the RBD region, further affecting its stability and function. Docking analysis of RBD variants and wild-type strains revealed that these variants have a higher affinity for the ACE2 (angiotensin 2 altered enzymes) receptor. Molecular interaction with CR3022 antibody revealed that binding affinity was less in comparison to wild type, with B.1.617 showing the least binding affinity.
Conclusions: The results of the extensive simulations provide novel mechanistic insights into the conformational dynamics and improve our understanding of the enhanced properties of these variants in terms of infectivity, transmissibility, **neutralization** potential, virulence, and host-viral replication fitness.

1. Introduction

COVID-19, a serious and continuously spreading pandemic affecting the world, creates severe ailments and apparently everlasting health problems. A few vaccines have exhibited potential & defensive effects upon COVID-19, mostly targeting the trimeric spike glycoprotein, which is involved in host cell interaction and gives passage to cell entry as well as the essential target for neutralizing antibodies. Essentially those were aimed against the earlier SARS-CoV-2 strain that emerged in 2019 in Wuhan China [1,2]. Due to the perceived ease of transmission and expansive mutations in spike proteins, the speedy evolution of new variants of SARS-CoV-2 is of high concern. B.1.1.7 (Alpha), B.1.351 (Beta), P.1 (Gamma), and B.1.617.2 (Delta) are SARS-CoV-2 variants of concern (VOC) strains, whereas B.1.617.1 (Kappa) and B.1.617.3 (Delta+) are SARS-CoV-2 variations of interest (VOI) strains, according to the World Health Organization (VOI).

The SARS-CoV-2 B.1.617 lineage, which was initially discovered in India, has spread around the world. B.1.617.1, B.1.617.2, and B.1.617.3 are the three sublineages that make up this lineage. Within the RBD of the S protein, mutations are identified in all of the sublineages [3]. The fast spread of the B.1.617 variation in India is thought to be related to the presence of several critical point mutations in the RBD, which may be enhancing the virus's cellular entrance, allowing it to infect a wider spectrum of target cells [4]. These alterations are also said to be the primary cause of their improved immune evasion ability. There are eight mutations in the SARSCoV-2 S protein of the B.1.617.1 (kappa) variant [5]. Seven of the eight mutations are found in the S1 region, while one is found in the S2 subunit. Two mutations in the RBD (L452R, E484Q), the area important for viral entry, are present in this variation. It was noted that several mutations of the receptor-binding domain (RBD), are essential for the interaction of Human angiotensin 2 altered enzymes (ACE2) [6] and antibodies, as well as region that neutralizes antibodies. The *in-silico* investigation revealed thatACE2 and potential antibodies

bind in a similar area on the spike protein [7,8] An antibody becomes very effective when forestalling viral spread by impeding the ACE2 binding site in the RBD. CR3022 antibody showed the most elevated binding affinity with SARS-CoV-2 protein RBD [9,10].

Here, in this study, we retrieved 28 different spike protein variants, and out of these 28 variants, 12 variants belong to the RBD region only. Here, we focused to know the impact of B.1.617 RBD variants that affect the interaction of CR3022 Abs and ACE2R to bind with the SARS-CoV-2 RBD as compared to others RBD variants and used molecular dynamics (MD) simulations to understand the conformational dynamics.

2. Materials and methods

2.1. Retrieval of crystal structures

Crystal structures of spike protein (PDBID-7AD1), ACE2 (PDBID-6ACG) and antibody CR3022 (PDBID 6YLA) were retrieved from PDB RCSB (https://www.rcsb.org/). All water molecules and hetero-atoms were removed by using Discovery studio visualization software (BIO-VIA 2020). (http://accelrys.com/products/collaborative-science/biovia -discovery-studio/visualization- download.php).

2.2. Homology modeling and energy minimization

Based on high similarity, 7AD1 (crystal structure of SARS-CoV-2) was selected as template for homology modeling of RBD mutant variants using the SWISS-MODEL [11]. Energy minimization and structural analysis of RBD mutant variants were done with UCSF Chimera [12]. Evaluation of the modeled structure was done by PDB-Sum (http://www.ebi.ac.uk/thornton-srv/databases/cgi-bin/pdbsum/GetPage.pl?pdbcode=index.html).

2.3. Docking analysis

Docking of RBD mutant variants with selected targets (ACE2 receptor and antibody structure CR3022) was carried out by PatchDock server [13] by choosing parameter RMSD esteem 4.0 and complex type as default. Docking investigation was based on geometric shape complementarities score. Higher score indicates higher binding affinity. Outcome of the results is based on the docking scores and interaction at the RBD regions. Protein-protein and antibody-protein interactions were visualized by LigPlot plus v2.2 [14].

Molecular interactions of antibody CR3022 and ACE2 receptor with RBD variants were performed by antibody script under antibody loop numbering scheme i.e. KABAT Scheme and DIMPLOT script algorithm package built into LigPlot plus v2.2 respectively.

2.4. Molecular dynamics simulation

The equilibrium and the dynamic behavior of wild and mutant variants of RBD Spike protein was studied by using GROMACS [15, 16]. MD simulation brings about time-dependent conformational changes and adjustment of protein, which opens to the alteration in unique nature after establishment of mutation in protein. We used GROMOS96 54a7 force field [17] for MD simulation study. We added solvent water around protein to facilitate from spc216.gro as a non-exclusive equilibrated 3-point dissolvable water model in a dodecahedron. Here, we kept the protein in the centre at least 1.0 nm from the case edges.

Further, the steepest descent algorithm was utilized for energy minimization, to remove the steric conflicts and unstable conformations. Further we equilibrate the system via NVT ensemble (constant Number of particles, Volume and Temperature) and NPTensemble (constant Number of particles, Pressure and Temperature). After achieving equilibrium process, we moved for MD run to 10ns.Data analysis was done by Gromacs tools i.e. gmx rms for RMSD (Root Mean Square Deviation),

gmxrmsf for RMSF (Root Mean Square Fluctuation), gmx gyrate for radius of gyration (Rg), gmxhbond for H-bond (for intra-protein H-bonds and for H-bonds between protein and water), and gmxsasa for SASA (solvent accessible surface). We further used GRACE software for data visualization.

3. Results

3.1. Docking analysis

We retrieved 28 variant mutants (S1) in spike protein identified to date. We found 12 variants/mutants in the RBD region. The RBD region is important for ACE2 and Antibody interactions. A few RBD variants have already shown to affect the vaccine efficacy as documented earlier by wet lab and dry lab results (S2 Table), however, the vaccine efficacy against the B.1.617 and K417G variants is yet to be elucidated. We have done structural analysis of all 12 RBD mutant variants and compared them with wild type. We found that seven mutant variants (F486L, Q493N, B.1.617 (L452R & E484Q), R408I, L455Y, K417G and E484K) have structural changes in RBD region (S3). We analyzed interactions between RBD variants and ACE2 receptor. Moreover, we also checked the interactions between antibody and RBD variants. We found that seven structurally changed variants (F486L, Q493N, B.1.617, R408I, L455Y, K417G and E484K) have high docking score against ACE2 receptor compared with wild type and less docking score against antibody (CR3022) unlike wild type (Table 1). Out of seven variants, B.1.617 (B.1.617 <Q493N < E484K < K486L < L455Y < R408I < K417G) demonstrates lowest binding energy against antibody. Molecular interactions of antibody and ACE2 receptor with RBD variants are depicted in S4–S5. Our *in-silico* study suggests that the B.1.617 and K417G variants may affect vaccine efficacy.

3.2. Molecular dynamics (MD) simulations

To examine the dynamic behavior, MD simulation runs for 10 ns to contemplate the structural stability of RBD mutant variants (F486L, Q493N, B.1.617, R408I, L455Y, K417G and E484K) in comparison to wild type. Various parameters studied all through the simulation trajectory, such as RMSD, Rg, RMSF, SASA, total number of intra-molecular hydrogen bonds of protein and H-bond between protein and water with the time dependent function of MD to examine the functional and structural impact of a mutant on wild protein.

The RMSD and RMSF (Figs. 1 and 2) of C-alpha chain atoms of all RBD mutant variants showed significant fluctuations in stability as well as in flexibility in comparison to Wild one. Rg and SASA analysis

Table 1

Prediction of RBD based variants interaction with antibody and ACE2 receptor.

| Interaction between Ab (CR3022_6YLA)-RBD_variants	Docking score	Interaction between ACE2-RBD_variants	Docking score	Structure hampered in the region of RBD
F486L	18,538	F486L	19,150	YES
B.1.617 strain (L452R &E484Q)	17,370	B.1.617 (L452R &E484Q)	18,434	YES
Q493N	17,722	Q493N	17,814	YES
R408I	18,984	R408I	17,656	YES
L455Y	18,758	L455Y	19,032	YES
K417G	19,428	K417G	18,734	YES
E484K	17,848	E484K	18,014	YES
A570D	20,342	A570D	17,856	NO
N501T	20,286	N501T	17,602	NO
N501Y	21,498	N501Y	17,600	NO
Q498Y	22,218	Q498Y	17,102	NO
N439K	20,556	N439K	17,174	NO
Spike_Wild	21,050	Spike_Wild	17,910	NO
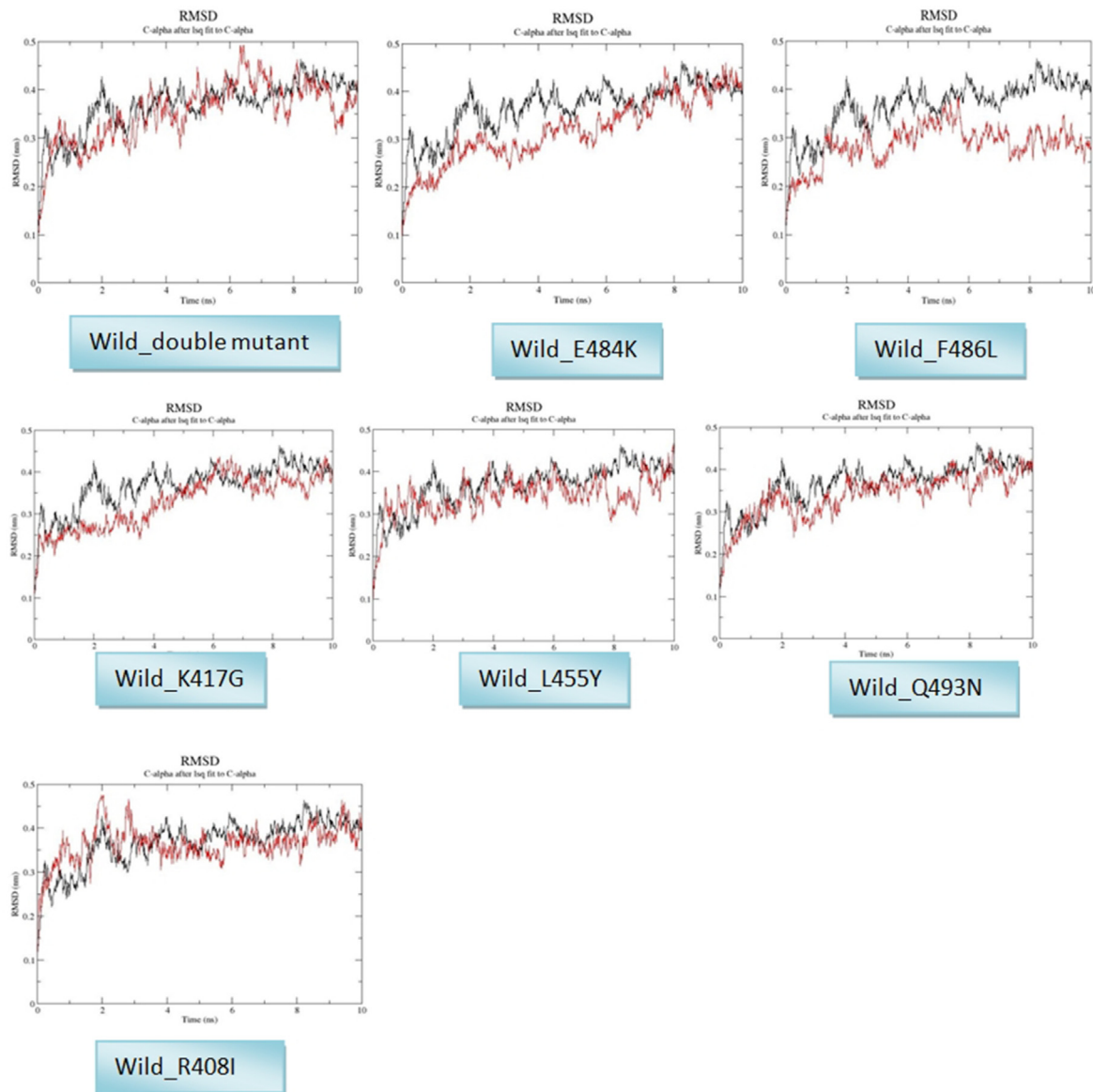
Fig. 1. RMSD analysis of RBD mutant variants along their wild type over 10 ns simulation. Black dot plot showed wild type whereas red indicates mutant.

(Figs. 3 and 4) depicted much fluctuation between mutant and wild type. The fluctuations of hydrogen bonds are easily shown in all RBD mutant variants as compared to wild type (total intra-molecular H-bond and H-bond between protein and water) (S6, S7 & S8 Table). These molecular simulation data showed hampered structural stability and complexity of all seven RBD mutant variants as compared to wild type.

4. Discussion

The newly emerged variants make our fight against global pandemic tougher as these variants might provide an evolutionary advantage, are more transmissible and harder for immune systems to detect. The 20I/501Y.V1 variant of the lineage B.1.1.7, first discovered in the UK, has eight major mutations in the spike genes that may affect vaccine efficiency, antibody therapy, and pose a threat of re-infection. In addition to remaining susceptible to antibody neutralization, the B.1.1.7 (alpha) variant does not seem to be a major burden for available vaccines [18,19]. B.1.351 (Beta variant), a variant first encountered in South Africa, is of greater concern as this variant is incompliant to NTD mAbs neutralization, mainly due to E484K mutations. In addition, we have also evidence that B.1.351 was more opposing to neutralization by convulsive plasma (9.4-fold) and vaccinated sera (10.3–12.4-fold) [20]. The SARS-CoV-2 P.1, the Brazilian variant of B.1.1.28 lineage, has 10 mutations in spike gene viz. D614G, T20N, D138Y, L18F, R190S, and P26S in the NTD and K417T, E484K and N501Y in the RBD region and H655Y within the furin cleavage site. It shares mutations similar to B.1.35. P.1 on the same 3 RBD residues which are resistant to neutralization by the RBD targeted mAbs. Shared E484K mutation is the main culprit, which emerged in more than 50 lines independently along with B.1.526, recently identified in New York. A significant loss of neutralizing activity has been shown by vaccinated serum and convalescent plasma towards P.1, but the decrease is not as good as compared to what was found against
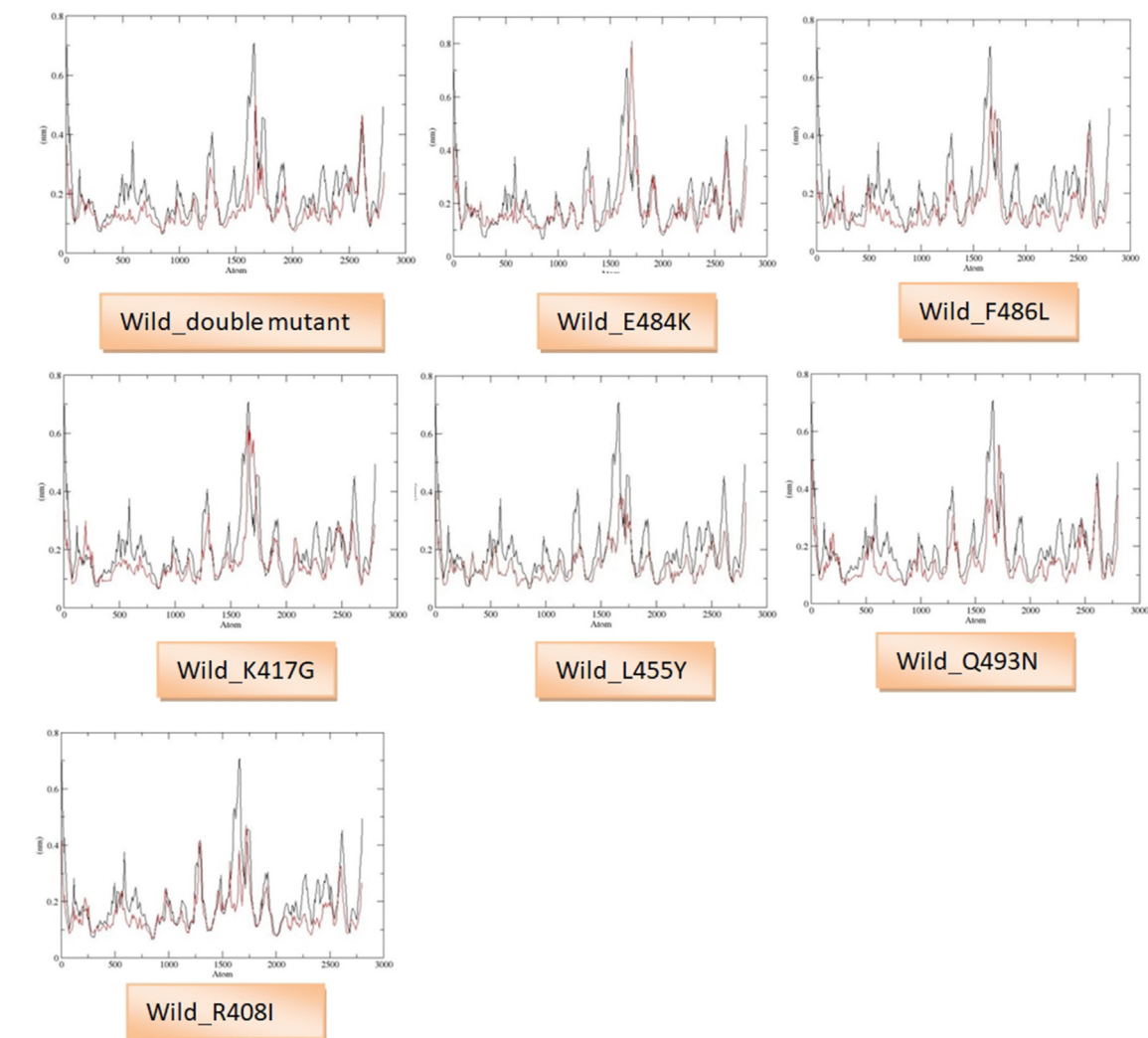
Fig. 2. RMSF analysis of RBD mutant variants along their wild type over 10 ns simulation. Black dot plot showed wild type whereas red indicates mutant.

B.1.351, Accordingly, the risk of re-infection by P.1 or dropped efficacy of vaccine protection may not be severe like B.1.351 [21]. The mRNA-1273 vaccine's neutralizing activity towards number of variants like B.1.351, B.1.1.7 + E484K, B.1.1.7, P.1, B.1.427/B.1.429, D614G, 20A.EU2, 20E [EU1], N439K-D614G, and previously identified mutant in Denmark mink cluster 5 were identified and found to have the same neutrality level as Wuhan-Hu-1 (D1414) [22]. Limited loss in antibody neutralizing activity against B.1.1.7 while significant loss against B.1.35 was shown by the AstraZeneca ChAdOx1 vaccine, thus maintaining its efficacy towards B.1.1.7 and demonstrating a major loss of efficacy against the benign version of B.1.151. Although the efficacy against B.1.1.7 was found to have retained by the BNT162b2 Pfizer/BioNTech COVID-19 vaccine. The Novavax vaccine (NVX-CoV2373) reported differential protective immunity in the clinical trials i.e. 96%, 60%, and 86% against parental strain, B.1.351 and B.1.1.7, respectively [23].

The consequences of the current examination propose that the new B.1.617 (along with 6 others RBD mutant strain) inside the receptor-restricting site could lessen the immunization adequacy and higher the probability of reinfections by influencing the SARS-CoV-2 connec-tion with the CR3022 antibody and ACE2 receptor. Our docking analysis observed that the binding affinity of mutant strain with ACE2 receptor is increased and is low with antibody compared to other variants.

The RMSD, RMSF, Rg, SASA, Intramolecular H-bond and H-bond between protein and water molecules were plotted to analyze the stability as well as flexibility of structurally hampered mutant RBD variants. Comparison of wild with mutant RBD protein, significant RMSD fluctuations were observed in all variants (B.1.617 0.25–0.5 nm, E484K nm 0.25–0.4 nm, F486L 0.2–0.3 nm, K417G 0.2–0.4 nm, L455Y 0.2–0.4 nm, L455Y 0.2–0.4 nm, Q493N 0.2–0.4 nm and R408I 0.25–0.45 nm). However more fluctuation was observed in B.1.617 as compared to others. The RMSD output showed that the protein stability could be influenced. We observed lower RMSF values of variant in comparisons to wild that confirms the compressed behavior of mutant trajectory. Higher value of Rg was noticed in all mutants' cases which indicate the possibility of lower compactness of protein. High fluctuations of SASA revealed that the protein structure and consequently protein function might be hampered. Fluctuations of total intra-molecular H-bond and H-bond between protein and water have been found in all structurally
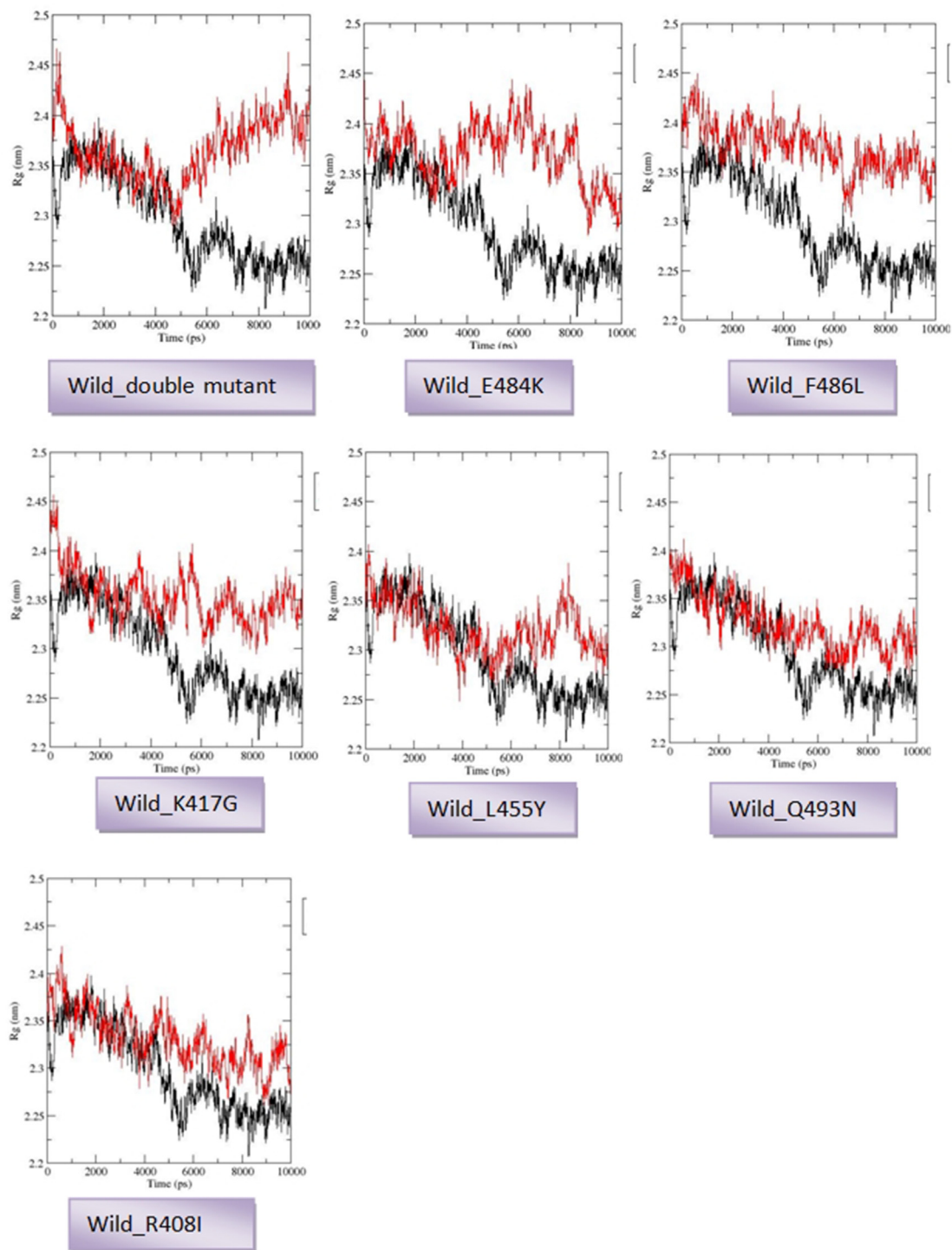
Fig. 3. Rg analysis of RBD mutant variants along their wild type over 10 ns simulation. Black dot plot showed wild type whereas red indicates mutant.

hampered RBD mutant variants which signify the rigidity of protein might be influenced [24].

Previous study has disclosed that the residues F486, L455, Q493, and N501 in the RBD spike protein form a major binding domain for the human ACE2 receptor [25]. A few mutants' viz.L455Y, Q493N, R408I, Q498Y, F486L, N501T within the RBD region (319–591), and D936Y& A930V within HR1 site (912–984) have also been studied by *in silico* analysis to investigate the basic structure of spike glycoprotein. After comparing MD simulations in mutants and WT, a significant destabilizing outcome of mutations on the HR1 and RBD domains was revealed. Researchers revealed compromised stability of the overall spike protein structures by investigating the effect of framed mutations, before binding to the receptor [26].
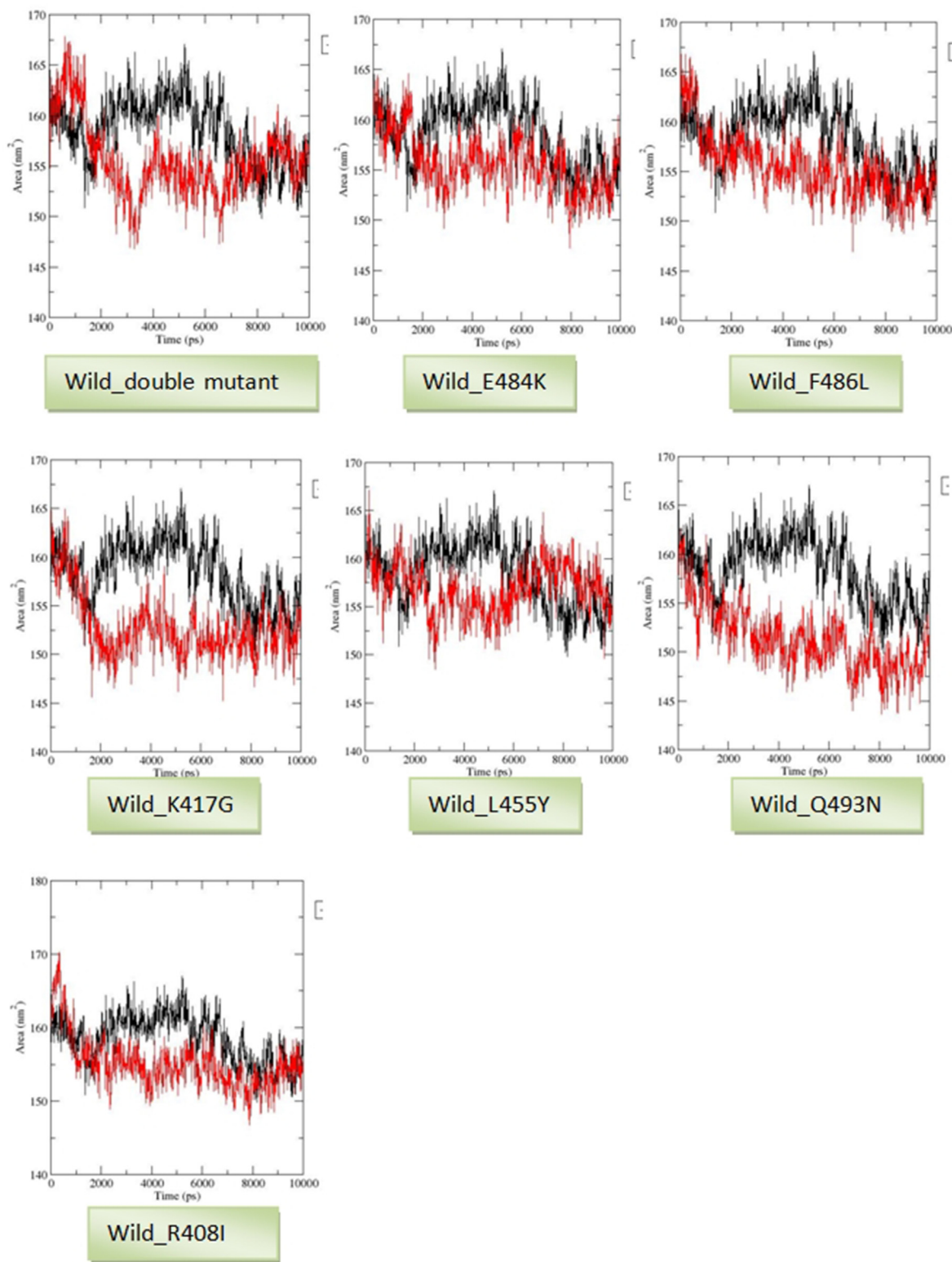
Fig. 4. SASA analysis of RBD mutant variants along their wild type over 10 ns simulation. Black dot plot showed wild type whereas red indicates mutant.

5. Conclusion

In this study, we performed molecular docking and simulation-based screening of B.1.617 and previously reported RBD variants of COVID-19 to compare the binding and functional stability of spike proteins. Results of the present study suggest that the B.1.617 strain and K417G within the receptor-binding site could reduce the vaccine efficacy and increase the chances of reinfection by affecting the SARS-CoV-2 interaction with the CR3022 antibody and ACE2 receptor. We have examined the impact of B.1.617 and earlier reported RBD variants on the spike glycoprotein's structural stability by *in silico* analysis along with molecular simulation data and found structural alteration in the RBD domain in seven mutant variants. Further molecular interaction study of CR3022 antibody and ACE2 receptor with the RBD variants and comparison with wild type

strain revealed the reduced binding affinity of all seven mutant versions with antibody, besides B.1.617 found to have the lowest affinity among all the RBD variants. These findings infer the possibilities of antigenic drift, ensuing incompatibility of current vaccine for B.1.617 strain. This information can be further harnessed for improvement of the available vaccines and aid in vaccine development. However, the results must be taken with caution as more research is still needed to explicate the exact consequences of the B.1.617 strain of SARS-CoV-2.

Author contributions

PR: Conceptualize and performed the experiments, data analysis, and writing-original draft. N: writing-original draft, and data analysis. CD: Data analysis and reviewing-original draft. GJ: reviewing-original draft and data analysis. CBM: reviewing-original draft and data analysis. PD: conceptualization, supervision, and reviewing-original draft. All authors contributed to the article and approved the submitted version.

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Declaration of competing interest

No Conflicts of interest to declare.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijmmb.2022.03.009.

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