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Bioinformatics analysis of SARS-CoV-2 RBD mutant variants and insights into antibody and ACE2 receptor binding

Prashant Ranjan[1], Neha[1], Chandra Devi[1]and Parimal Das[1]

[1]Centre for Genetic Disorders, Institute of Science, Banaras Hindu University, Varanasi-221005, India
Email: parimal@bhu.ac.in

Abstract

Prevailing COVID-19 vaccines are based on the spike protein of earlier SARS-CoV-2 strain that emerged in Wuhan, China. Continuously evolving nature of SARS-CoV-2 resulting emergence of new variant/s raise the risk of immune absconds. Several RBD (receptor-binding domain) variants have been reported to affect the vaccine efficacy considerably. In the present study, we performed *in silico* structural analysis of spike protein of double mutant (L452R & E484Q), a new variant of SARS-CoV-2 recently reported in India along with K417G variants and earlier reported RBD variants and found structural changes in RBD region after comparing with the wild type. Comparison of the binding affinity of the double mutant and earlier reported RBD variant for ACE2 (angiotensin 2 altered enzymes) receptor and CR3022 antibody with the wild-type strain revealed the lowest binding affinity of the double mutant for CR3022 among all other variants. These findings suggest that the newly emerged double mutant could significantly reduce the impact of the current vaccine which threatens the protective efficacy of current vaccine therapy.

Keywords: SARS-CoV-2, double mutant, immunology, computational biology, *in silico*, structural biology, vaccine efficacy, antiviral therapy

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Introduction

COVID-19, a serious and continuously spreading pandemic affecting the world, creates severe ailments and apparently everlasting health problems. Possibilities of a windup of this outbreak are developing adequate interventions. While Monoclonal antibody (mAb) therapy has gained emergency use approval; 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 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.(Korber *et al*., 2020; Chen *et al*., 2020). 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. It was noted that several mutations of the receptor-binding domain (RBD), are essential for the interaction of Human angiotensin 2 altered enzymes (ACE2) (Yan *et al*. 2020) and antibodies, as well as region that neutralizes antibodies. It is therefore imperative to understand up to what extent mutations interrelated with SARS-COV-2 affect the vaccination. Taking these into account both vaccination as well natural infections, several reports deal with the outcome of these variants on antibody ligation and function. The RBD can exist in two conformities, alluded to as "up" and "down" i.e. receptor accessible and receptor in-accessible (Wrapp *et al*. 2020). The *in silico* investigation revealed that ACE2 and potential antibodies binds in a similar area on the spike protein (Hwang *et al*. 2006, Sui *et al*. 2004). An antibody becomes very effective when it forestalling viral spread by impeding the ACE2 binding site in the RBD. CR3022 antibody showed the most elevated binding affinity with SARS-CoV-2 S-protein RBD (Hussain *et al*. 2020, Huo *et al*. 2020).

Based on the up to date literature survey, we retrieved 28 different spike protein variants and out of these 28 variants, 12 variants belong to RBD region only. Here, we report RBD variants that affect the ability of CR3022 and ACE2 to bind with the SARS-CoV-2 RBD through *in silico* analysis.

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Material and methods

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 hetro-atoms were removed by using Discovery studio visualization software (BIOVIA 2020). (http://accelrys.com/products/collaborative-science/biovia-discovery-studio/visualization- download.php).

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 (Lyskov and Gray 2008). Energy minimization and structural analysis of RBD mutant variants were done with UCSF Chimera (Pettersen *et al*. 2004). Evaluation of the modeled structure was done by PDB-Sum.

Docking analysis

Docking of RBD mutant variants with selected targets (ACE2 receptor and antibody structure CR3022) was carried out by PatchDock server (Ranjan *et al*. 2020) by choosing parameter RMSD esteem 4.0 and complex type as default. Docking investigation was based on geometric shape complementarity 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.

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.

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Results

We have retrieved 28 variant mutants **(Figure1)** in spike protein from literature survey. We found 12 variants/mutants in RBD region. RBD region is important for ACE2 and Antibody interactions. We have done structural analysis of all 12 mutant variants and compared with wild type and found seven mutant variants (F486L, Q493N, double mutant (L452R & E484Q), R408I, L455Y, K417G and E484K) have structural changes in RBD region **(Figure2)**. We analyzed interactions between RBD variants and ACE2 receptor. Moreover, we checked the interactions between antibody and RBD variants too. It was found that seven structurally changed variants (F486L, Q493N, Indian double mutant (L452R & E484Q), 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 **(Table2)**. High docking score signifies high binding affinity and low docking score signifies low binding affinity. Out of seven variants, double mutants (Double mutant<E484K<K486L<L455Y<R408I<K417G) have lowest binding energy against antibody. Molecular interactions of antibody and ACE2 receptor with RBD variants are depicted in **supplementary file 1.** A few RBD variants already shown to affect the vaccine efficacy as documented earlier by wet lab and dry lab results **(Table1)**, however, the vaccine efficacy against the double mutant and K417G variants is yet to be elucidated. Our *in silico* study suggests that the double mutant and K417G variants may severely affect the vaccine efficacy.

Discussion

"Double mutant" coronavirus variation with a combination of changes not seen in any other places in the world than in India according to Times of India and BBC news. Many SARS-CoV-2 variants have been detected in the last few weeks. 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 the threat of re-infection. In addition to remaining susceptible to antibody neutralization, the B.1.1.7 variant does not seem to be a major burden for available vaccines (Shen *et al*., 2021; Muik *et al*., 2021). B.1.351, a variant first encountered in South Africa, is of greater concern that this variant is incompliant to NTD mAbs neutralization, mainly due to E484K mutations. In addition, B.1.351 was more opposing to neutralization by convulsive plasma (9.4-fold) and vaccinated sera (10.3–

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12.4-fold) (Wang *et al*., 2021). 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 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 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 (Wang *et al*., 2021).
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) (Wu *et al*., 2021). 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 (Tarke *et al*., 2021).

A 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 (Wan *et al*., 2020). A few mutants viz. L455Y, Q493N, R408I, Q498Y, F486L, N501T within the RBD region (319-591), 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

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investigating the effect of framed mutations, before binding to the receptor (Ahamad *et al*., 2020).

Conclusion

The results of the present *in silico* study suggest that the new Indian strain double mutant and K417G within the receptor-binding site could reduce the vaccine efficacy by affecting the SARS-CoV-2 interaction with the CR3022 antibody and ACE2 receptor. We have examined the impact of double mutants and earlier reported RBD variants on the spike glycoprotein's structural stability by *in silico* analysis 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 double mutant with antibody, besides double mutant 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 double mutants Indian strain. This double mutant strain seems to be a major burden for the available vaccine that could reduce the vaccine efficacy drastically and so may increase the chances of re-infection. However, more research is still needed to explicate the exact consequences of the double-mutant strain of SARS-CoV-2.

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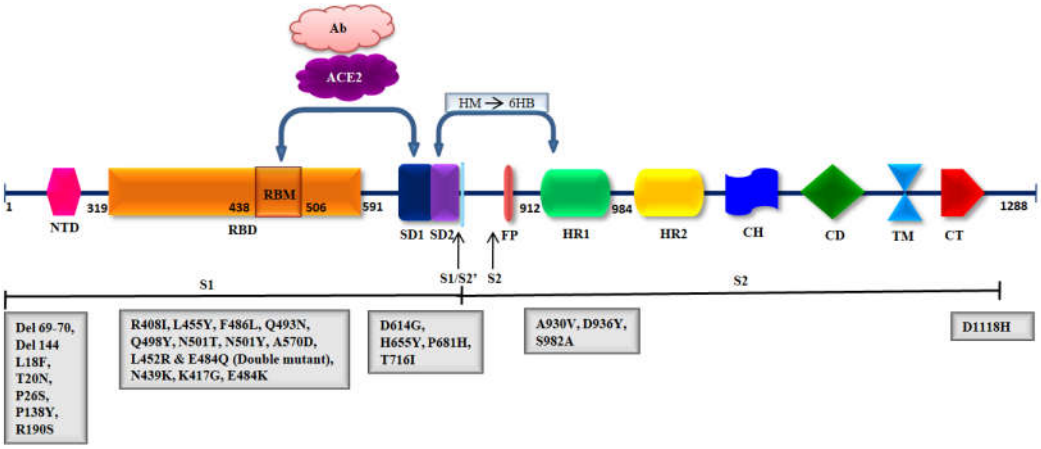
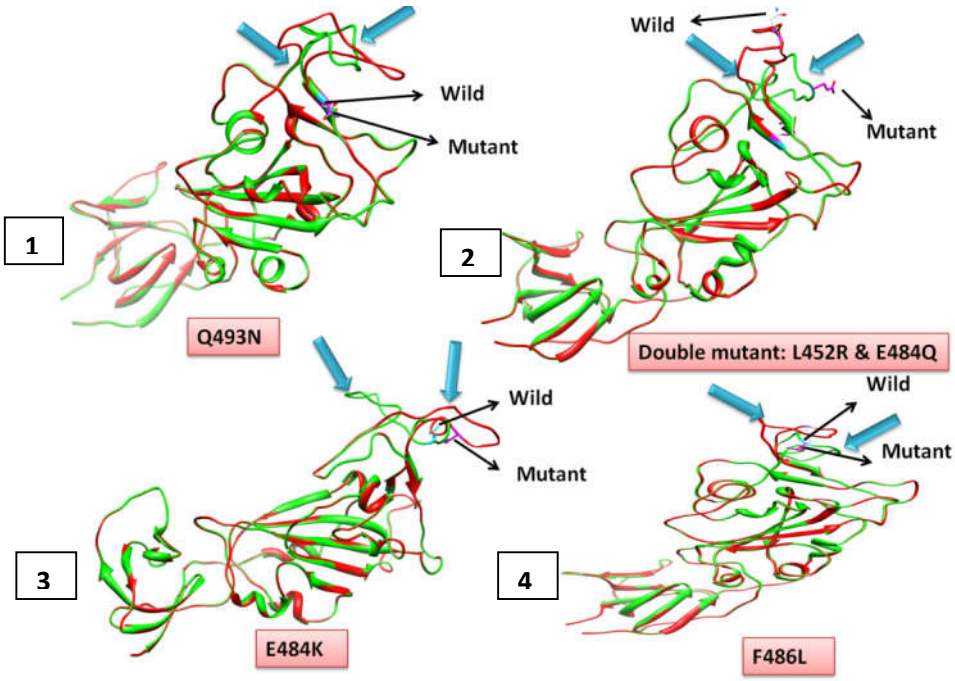


Figure1. Schematic representation of SARS-CoV-2 spike glycoprotein along with depiction of ACE2 and Antibody binding on RBD.

NTD: N-terminal domain, RBD: receptor-binding domain, RBM: receptor-binding motif, SD1 and SD1: Subdomain1 and 2, S1 and S2: Protease Cleavage Site 1 and 2, FP: fusion peptide, HR1 and HR2 heptad-repeat regions 1 and 2, CH: Central Helix, CD: Connector Domain, TM transmembrane region, CT cytoplasmic tail, HM: Homotrimeric assembly, 6HB: Six Helix bundle.



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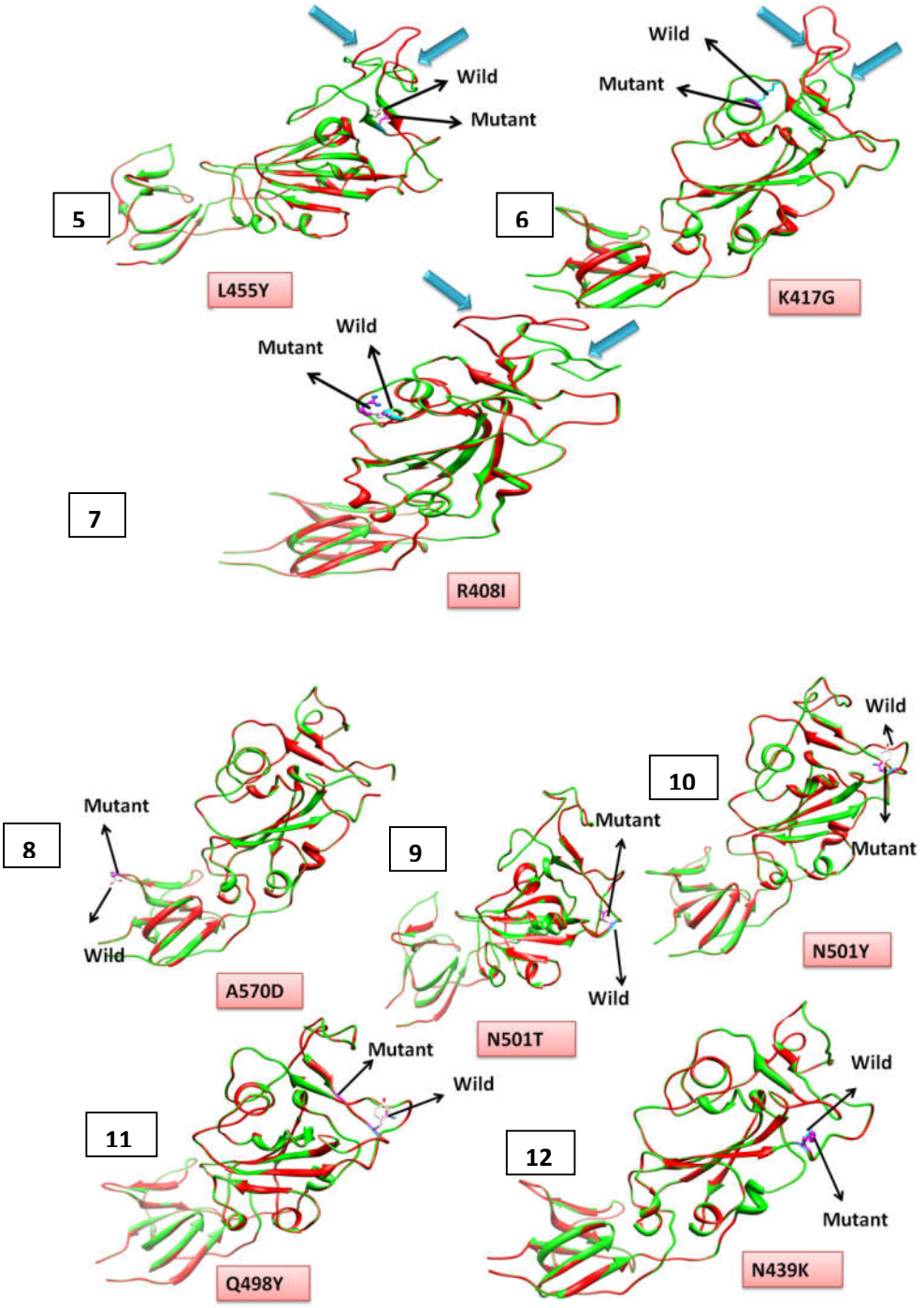

Figure2. Structural superposition of RBD based variants with wild type: 1-7 represents seven RBD mutant variants depicting structural changes when compared with wild type. 8-12 shows five RBD mutant variants do not have changes on RBD region. Green color indicates wild type and red color indicates mutant.

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Table1. Table showing studies related to vaccine efficacy hampered by RBD based variants detected by different methodology.

Mutant Strain	Method of assessment	Finding	Reference
N501Y, A570D, N439K	Phenotype accessing by convalescent sera, mAB, serum sample from phase-I trail of mRNA-1273 vaccine (Moderna) & NVX-CoV2373 (Novavax)	Remains sensitive to neutralization, unaffected to current vaccine	Shen *et al.*, 2021
E484K	By convulsive plasma and vaccinated sera	Reduced neutralization, hampers vaccine efficacy	Wang *et al.*, 2021
Q498Y, Q493N, L455Y, F486L, N501T	Computation Algorithms & MD Simulation	Destabilized RBD & HR1 domain of Spike, affecting receptor binding site	Ahamad *et al.*, 2020
R408I (B.1.1.7)	*In silico* approach by Informational Spectrum Method	RBD binding with ACE2 got affected, resistance to vaccine based on wild type SARS-CoV-2	Veljko *et al.*, 2020
K417G	NA (Not Available)	NA	NA
Double mutant (L452R & E484Q)	NA	NA	NA

Table2. 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	18538	F486L	19150	YES
Double mutant (L452R & E484Q)	17370	Double mutant (L452R & E484Q)	18434	YES
Q493N	17722	Q493N	17814	YES

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R408I	18984	R408I	17656	YES
L455Y	18758	L455Y	19032	YES
K417G	19428	K417G	18734	YES
E484K	17848	E484K	18014	YES
A570D	20342	A570D	17856	NO
N501T	20286	N501T	17602	NO
N501Y	21498	N501Y	17600	NO
Q498Y	22218	Q498Y	17102	NO
N439K	20556	N439K	17174	NO
Spike_Wild	21050	Spike_Wild	17910	NO