Major structural deviations in the receptor binding domain of SARS-CoV-2 spike protein may pose threat to the existing vaccines.

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The current vaccines designed against the wild type SARS-CoV-2 offer great protection if taken according to the recommended dosages at recommended time periods. However, there have been several mutant strains of the virus that posed a threat to currently existing therapeutics. These mutant strains may not necessarily fail the current vaccines but eventually may due to the evolution of more mutant variants. In order to understand the epitope variation we performed a computational biology-based study here in which systematic amino acid substitutions were modeled at selected positions in the receptor binding domain (RBD) of the viral spike protein that interacts with the human angiotensin converting enzyme-2 (ACE-2) receptor. Out of 11 positions, four (K89, Y121, N173 and G174) exhibited significant epitope topology changes as big as >4 Å in the C-alpha compared to the wild type RBD suggesting that they can pose threat to the existing vaccines. Currently these mutants are under critical evaluation to assess the extent of damage that they can cause to the existing vaccines.

Key words: COVID-19, Coronavirus, spike protein, antigen, epitope, structural deviation, vaccine.

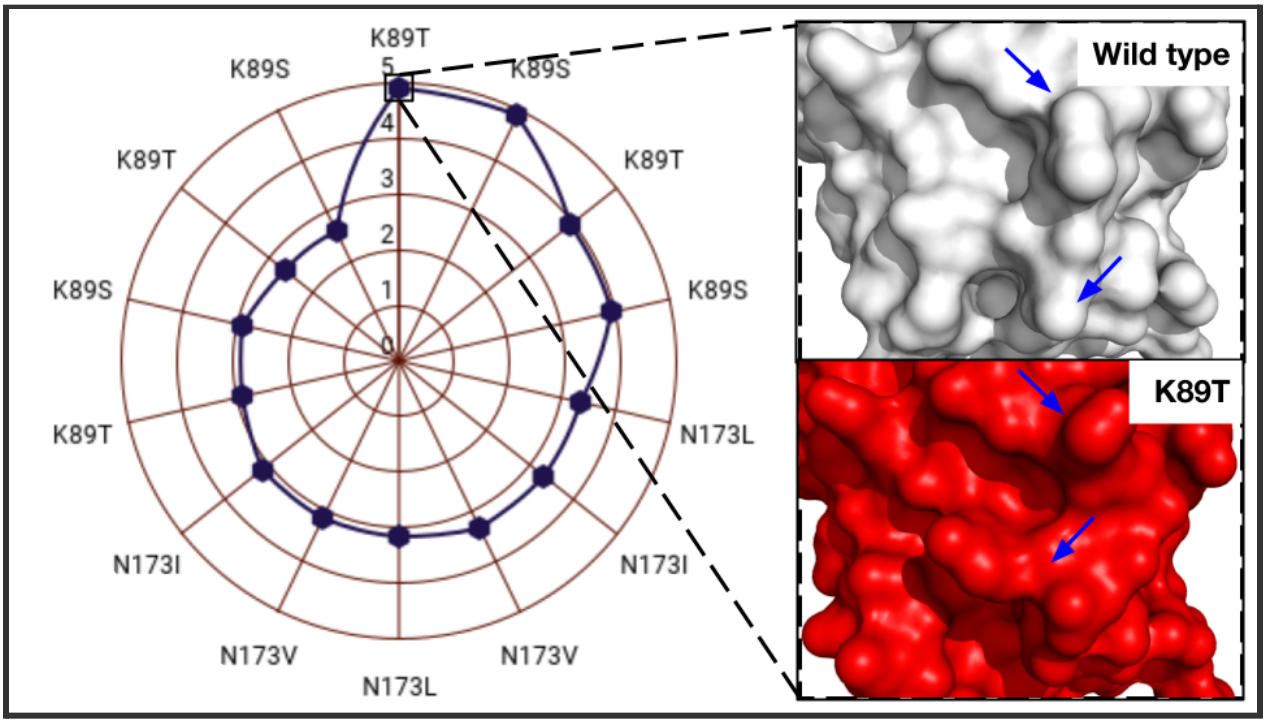

Figure 1. Mutations at K89 and N173 plotted with their high deviations from wild type in a circular plot (left). Highest deviation of the mutant K89T (4.9 Å), shown on the right side in comparison with the wild type. Blue arrows pointing to the structural deviations.

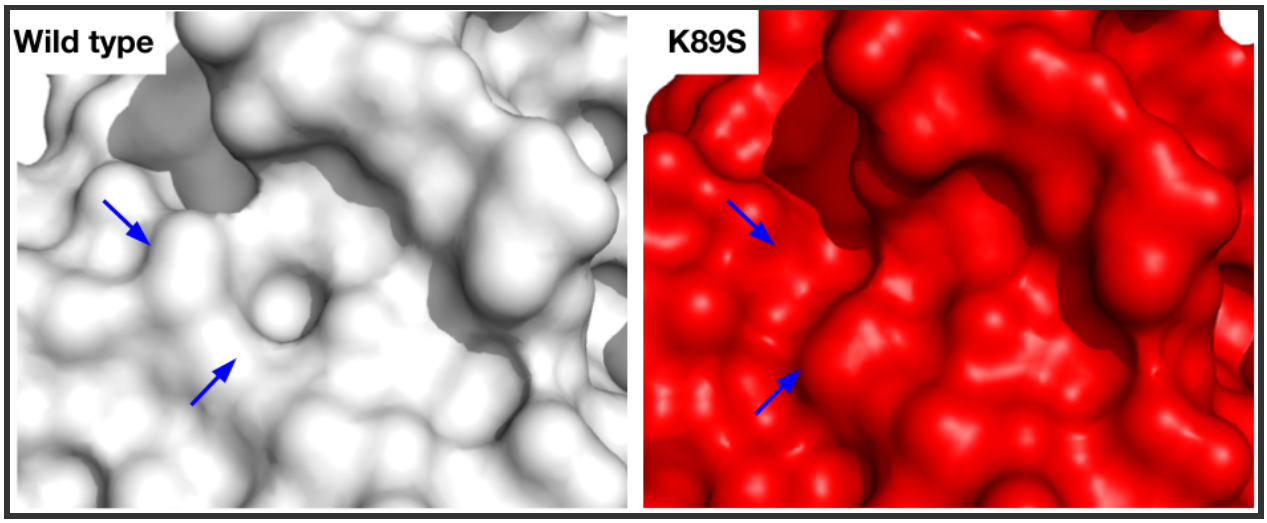

Figure 2. Off-site structural deviation in the mutant K89S (right) compared to the wild type (left).

Antigen shape is very critical in order to maintain the epitope topology for an antibody to continue its recognition. Mutations in the Severe acute respiratory syndrome coronavirus-2 (SRS-CoV-2) spike protein (1) may alter the epitope topology (2) that could be challenging for the currently used antibodies in passive immunization therapies and vaccines. In this study we made a systematic amino acid substitution study in 11 positions in the receptor binding domain (RBD) of the viral spike protein that were identified to be interacting with the human angiotensin converting enzyme-2 (ACE-2) (3) receptor. Out of these 11 positions, we identified four hotspots where mutations generated significant structural deviations. Our study found that the amino acid substitutions at K89, Y121, N173 and G174 yielded highest C-alpha deviations in the mutant models when compared to the wild type RBD. As shown in Figure 1, the 14 amino acid substitutions that resulted in the highest deviations were plotted to compare them. The C-alpha deviations ranged from 2 Å (K89M) to almost 5 Å (K89S/T) suggesting that these mutants could pose threat to the currently used vaccines that are mainly based on the wild type viral spike protein. At each of the 11 positions in the RBD, other than the wild type amino acid, the remaining 19 natural amino acids were substituted one at a time and the mutant models were prepared using SWISS MODEL. The models were then superposed onto the wild type RBD by using the LSQKAB program in the CCP4 suite of programs. Restrains were posed on the C-alpha atoms of both mutant and wild type models such that the true root mean square deviations (RMSDs) can be calculated instead of superimposing the models in various molecular graphics programs that may not accurately perform the task. During analysis, the RMSD values that are less than or equal to 1 Å were ignored which may contribute towards the protein dynamics. However, RMSD values above 1 Å were considered in this study. All the RMSD values that were either 2 Å or above were considered as significant deviations that alter the topology of the epitope. Utmost care was taken especially when evaluating the epitope topology changes in the antigen (spike protein RBD) because it is very critical that one should identify only true structural deviations. To this end, we have manually examined each RMSD value using the superposed models in a molecular graphics software. Amino acid substitutions at positions K89/N173 exhibited RMSDs > 3 Å (Figure 1) while the substitutions at positions Y121/G174 exhibited < 3 Å RMSDs.

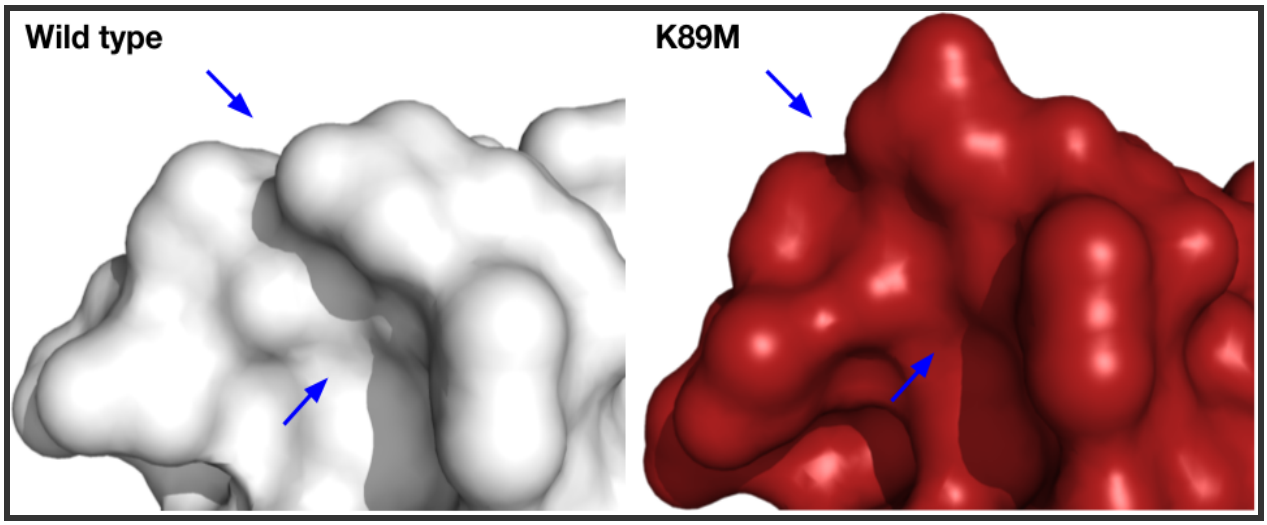

Figure 3. Off-site structural deviation in the mutant K89M.

Interestingly, some of the mutants showed structural deviations at the point of amino acid substitution within the RBD-ACE2-interface while others exhibited RMSDs in offsites (Figures 2 and 3) that may be allosterically controlled by the mutation in the RBD-ACE2-interface. We are currently in the process of building a training dataset for machine learning purposes using the full length SARS-CoV-2 spike protein with systematic amino acid substitutions along with the possible permutations and combinations to understand any structural deviations that could cause resistance against the currently used vaccines. The ongoing and upcoming research on the structural deviations leading to the changes in antigen topology and their effect on the current vaccines will be published in the future issues of TCABSE-J.

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Conflict of interest: The Analysis Report presented here is an ongoing project currently at TCABS-E, Rajahmundry, India. The authors invite collaborations without any conflict of interest.