Molecular Level Dissection of Critical Spike Mutations in SARS-CoV-2 Variants of Concern (VOCs): A Simplified Review

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SARS-CoV-2 virus during its spread in the last one and half year has picked up critical changes in its genetic code i.e. mutations, which have leads to deleterious epidemiological characteristics. Due to critical role of spike protein in cell entry and pathogenesis, mutations in spike regions have been reported to enhance transmissibility, disease severity, possible escape from vaccine-induced immune response and reduced diagnostic sensitivity/specificty. Considering the structure-function impact of mutations, understanding the molecular details of these key mutations of newly emerged variants/lineages is of urgent concern. In this review, we have explored the literature on key spike mutations harbored by alpha, beta, gamma and delta ‘variants of concern’ (VOCs) and discussed their molecular consequences in the context of resultant virus biology. Commonly all these VOCs i.e. B.1.1.7, B.1.351, P.1 and B.1.617.2 lineages have decisive mutation in Receptor Binding Motif (RBM) region and/or region around Furin cleavage site (FCS) of spike protein. In general, mutation induced disruption of intra-molecular interaction enhances molecular flexibility leading to exposure of spike protein surface in these lineages to make it accessible for inter-molecular interaction with hACE2. A disruption of spike antigen-antibody inter-molecular interactions in epitope region due to the chemical nature of substituting amino acids hampers the neutralization efficacy. Simplified surveillance of mutation induced changes and their consequences at molecular level can contribute in rationalizing mutation’s impact on virus biology. It is believed that molecular level dissection of these key spike mutation will assist the future investigations for a more resilient outcome against severity of COVID-19.

1. Introduction

Severe Acute Respiratory Syndrome Corona Virus 2 (SARS-CoV2) which is causing agent of COVID-19 pandemic, is composed of genomic material (RNA) surrounded by a protective coat of protein.[6] Crown shaped spike protein on the envelope of SARS-CoV-2 is a multifunctional molecular machine responsible for host cell recognition followed by subsequent adaptations, binding and invasion.[7] Hence it acts as a key to enter human cell. Due to its critical role in infection initiation, spike protein has been considered as topmost target of neutralizing antibodies and drug molecules.[8] However, changes in the genome (genetic material) sequence due to mutation in different region of spike protein reduces the efficacy of existing vaccines and antiviral drugs which were developed for parent virus.[9,10] New variants may not behave as the previously characterized sequence (wild type/preexisting mutants) against recognizing markers/vaccines/therapeutics and may escape from diagnostic tests, immune system, therapeutic drugs.[11-15]

Mutation is a natural phenomenon that occurs during the replication cycle of all viruses and SARS-CoV-2 is no exception.[12] When SARS-CoV-2 latches onto host cells and subsequently uses the host cell’s machinery to multiply its genetic material, during replication process some newly generated virus/viron copy does not have same genome sequence (RNA sequence) as that of origin alone. From a chemist’s perspective, when an error or mutation occurs during replication, it results in change of nucleotide sequence/nucleic acid sequence. This change in nucleotide sequence manifests itself as change in amino acid sequence in the protein being synthesized using these new ‘sequence’ information. So, a simple change in genome sequence (nucleotide sequence) may result in the synthesis of a protein with different amino acid sequence.[13] Different deletion and substitution in amino acid sequence of spike protein cause significant alteration in its different properties like polarity, bond interactions etc. which in turn leads to change in its structure, stability and molecular surface character finally ending up in evolution of different variants of SARS-CoV-2.[14]

Since late 2019, when the original SARS-CoV-2 was first detected, several new variants of this virus have been reported. Many of these new variants with different genome sequence (such as B.1.1.7 (or 501Y.V1 or alpha), B.1.351 (or 501Y.V2 or beta), P.1 (gamma), B.1.617.2 (delta) etc.) have been found to be highly transmissible and thus spread rapidly.[15-18] Indeed
different genome sequences of some variants have underlined current diagnosis tests, antibody therapies or natural immunity since they are dependent on preexisting resistant mutants.\footnote{So far, only partial understanding of the molecular reasons leading towards enhanced infection and cellular uptake of new SARS-CoV-2 variants of concern (VOCs) like B.1.1.7 (alpha), B.1.351 (beta), P.1 (gamma) and B.1.617.2 (delta) lineages exists. Until detailed experimental data about structures of mutated spike protein of these new variants and their bound complexes becomes available, computational mutagenesis/simulation studies are the only asset to combat with these new variants of SARS-CoV-2.\footnote{25}} In order to come up with a countermeasure for these new variants, molecular level understanding of amino acid sequence alteration and consequent changes in the intra- and inter-molecular interactions is essential.\footnote{26–28} By molecular level evaluation and insight of possible impacts of genomic mutations on the virus functions can allow one to draw robust inferences which in turn can assist in developing a diagnostic kit, vaccine or immune resistance efficient against the new variants. Thus, molecular scale surveillance of new variants and their consequences is important in minimizing the threat of COVID-19 against human health.\footnote{19,20}

Determination of molecular consequences of mutations within newly emerging variants is challenging and time consuming especially with sub-10Å resolution. For visualizing intra- and inter-molecular interactions of spike protein of new variants (with differ genome sequence) at molecular scale, first X-ray crystallography and/or cryo-EM structures of mutated spike protein and their bound complex with receptor/antibody is required.\footnote{21} Most of the data obtained by X-ray crystallography and/cryo-EM have the amino acid sequences but hydrogen atom positions are not there.\footnote{17,22,23} Thus, subsequent computational studies on these structures are needed to provide fine details of interactions, binding affinity and underlying mechanisms of biological functions.\footnote{22,24,25} Such kind of detailed experimental-computational joint data are so far lacking for the new variants of SARS-CoV-2 quantifying their impact on the virulence. However, many computational investigations/simulations-analysis are available in literature where researchers have artificially mutated the standard wild type protein and then using this model they have examined the mutational consequences.\footnote{26–28} So far, only partial understanding of the molecular reasons leading towards enhanced infection and cellular uptake of new SARS-CoV-2 variants of concern (VOCs) like B.1.1.7 (alpha), B.1.351 (beta), P.1 (gamma) and B.1.617.2 (delta) lineages exists. Until detailed experimental data about structures of mutated spike protein of these new variants and their bound complexes becomes available, computational mutagenesis/simulation studies are the only asset to combat with these new variants of SARS-CoV-2.\footnote{25}

In the present article, repertoire of recent investigations focusing on the molecular consequences of spike mutations in variants of concern (VOCs) of SARS-CoV-2 [B.1.1.7 (alpha), B.1.351 (beta), P.1 (gamma) and B.1.617.2 (delta) lineages] have been carried out. It is aimed to compile the scattered literature of these VOCs with molecular scale insights. It is believed that consolidation of crucial information regarding molecular basis of SARS-CoV-2 spike protein at single place can help not only in expediting the coherent future efforts, also it can help in creating a correlated perspective between spike mutation and its consequent biological function. The article starts with general chemist view/approach about mutation, afterwards brief saga of mutations in highly pathogenic human sister Coronavirus (hCoVs) viz. SARS-CoV and MERS-CoV has been presented. Subsequently, focus of the article shifts on the WHO labelled variants of concern (VOCs) of SARS-CoV-2. In this context, special focus has been made on the:

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(i) Modification in the intra-molecular interactions in spike protein's polypeptide chain and its possible manifestation in conformational/structural changes due to chemical/physical restraints imposed by crucial missense mutation (point mutation in which a single nucleotide changes in genome leading to single amino acid substitution in polypeptide chain). Importantly in what manner the chemical nature of substituting amion (amino) acid (charged, neutral,polar, non-polar, acidic, basic, hydrophobicity, hydrophilicity etc.) dictates the chemical interactions.

(ii) Influence of key spike mutations on inter-molecular interactions involved between spike protein Receptor Binding Domain (RBD) and human cells angiotensin-converting enzyme 2 (hACE2) receptor during their binding as it is directly releated to cell entry and infectivity.\(^{24}\)

(iii) Impact of harbored crucial spike mutations on binding of spike antigen with neutralizing antibodies. Antibodies are immune molecules (protective protein molecule) produced by the immune system and they generally target specific segments (epitope) of spike proteins/antigens for neutralization of virus. Therefore, influence of mutation on spike antigen-antibody inter-molecular interactions is of great interest for development of vaccine and therapeutic antibodies against these VOCs.

Last section of article encompasses the generalized overview and perspective of molecular consequences of mutations in these VOCs. Throughout the article, information has been consolidated with lucid as well integrative chemical biology approach. Combining multiple lines of approaches allowed us to draw vital inferences. It is believed that amalgamated compendium of molecular consequences of spike mutation in VOCs of SARS-CoV-2 will help in creating a common consensus on the molecular mechanism and will guide the future investigations and will assist in combating the pandemic through appropriate intervention strategies. In assembling the literature, we have relayed principally on accredited information available in the peer reviewed journals for authenticity, however work reported in non-peer reviewed manuscripts available in pre-print, as well as other information publicly available on websites also have been cited for the sake of completion.

2. Mutation and their outcome in highly pathogenic human Coronaviruses (hCoVs)

In biology, information is stored in molecular sequence, therefore molecular dissection are is required to get the underlying intricacies. Genetic information is stored in nitrogen base sequence present in nucleic acid molecules [deoxyribonucleic acid (DNA) molecules/ribonucleic acid (RNA)]. Central dogma traces the flow of genetic information from DNA to protein (DNA→RNA→Protein). Instructions for making proteins with the correct sequence of amino acids are encoded in nucleotide sequence of nucleic acid and information needs to be transferred in detail, residue-by-residue. Any alteration in any of these nucleotide base sequence of nucleic acid manifest itself as change in amino acid sequence because a sequence of three nucleotides codon specifies a particular amino acid in a protein. So, a simple change in nucleotide base sequence may result in synthesis of a protein with slightly different structure and hence different properties like polarity, bond interactions etc. which may ultimately result in change of biological function.\(^{30}\) Mutation/changes can be of different type such as base insertion, base deletion and/or base substitution in nucleotide sequence.\(^{13}\) A point mutation in which a single base-change in nucleotide sequence results in a codon that encodes for a different amino acid is called missense mutation (change of a single amino acid in resultant protein). For example ‘E484K’ is a missense mutation where glutamic acid (E) has been substituted by lysine (K) at 484\(^{th}\) residue position in the resultant polypeptide chain/protein. Another mutation ‘AH69/ΔV70’ is a deletion mutation i.e. removal of amino acid residues 69 and 70 in the resultant polypeptide chain/protein.\(^{31}\)

Mutations (i.e. change in a genome base sequence) can result due to mistakes made during replication of DNA genome during cell division or outside factors such as exposure to ionizing radiations. Reliability of DNA genome replication is examined using polymerase proofreading and the mismatch repair pathway. The extent of proofreading in DNA genome replication determines the mutation rate, and is different in different species, including virus. If a virus has to replicate in a new host species, it must be able to adapt to the new host. The more genetically variable a virus, more it will be adaptable to the new host species and hence capable of producing new infections. The important of mutation can be realized by the fact that most of emerging viruses have RNA as genetic material instead of DNA. This is because the rate of mutation in RNA is many orders greater than that of DNA which gives them an evolutionary edge over their DNA counterparts.\(^{32}\) Thus, Mutations play an important role in the transmission of virus from one host species to another.

Mutant form of virus (single or multiple mutations) is called ‘variant’ as it has genetic sequence different from its parent. Variant carrying the mutation is genetically distinguishable from its predecessors (non-mutant relative or wild type), but its biological functions (like transmutability, pathogenicity, immunogenicity, virulence) may or may not significantly differ. In case when the variant is not only genetically distinguishable from its parent, but its biological behaviour/function is also differing significantly, then these types of variants are labelled as ‘strain’. In a strain, the genetic change in molecular sequence causes significant alteration in physical properties such as enhances the binding affinity to a particular receptor making the virus more transmissible than its predecessors leading to materially change in the viral epidemiology. Based on phylogenetic family tree nomenclature, these variants/strains are technically termed as “Lineage”. The change is lineage means movement to other branch of phylogenetic family tree.

Generally, two different lineages of evolution of coronavirus are known namely the avian lineage and the mammalian lineage.\(^{33}\) Avian lineage includes the gamma-coronavirus and delta-coronavirus whereas the mammalian lineage comprises the alpha-coronavirus and beta-coronavirus which can infect the mammals like bats, murine, bovine, humans, etc.\(^{34,35}\)
Ancestral beta-CoVs (Bat-CoV) were unable to infect humans as they cannot bind with human Angiotensin converting enzyme II (hACE2) situated in the lower respiratory tract of human, as a cell receptor for facilitating entry into cell. However, the emergence of the SARS-CoV in 2003 was due to mutations of CoVs in bats and civets that allowed virus to attach to the host cell receptor hACE2 in humans and infect. Further, the SARS-CoV has also undergone mutation and the estimated mutation rate was of the order $10^{-8}$ substitutions/nucleotide/cell infection. [35] Spike protein of SARS-CoV showed significant mutations in both S1 domain (domain which attached to the host ACE2 receptor) and S2 domain (which fuses with the host cell membrane to enter into the host cell). Moreover, 29 nucleotide sequences in the open reading frame 8 (ORF8) of SARS-CoV-2 genome (encoding responsible for the viral protein similar to ORF8 of bats and civets) got deleted during mutation and it was not found in the virus isolated from patients infected in later stages of outbreak. The 29 nucleotide deletion in ORF8 region of the virus resulting in the formation of two segments ORF8a and ORF8b is widely accepted as one of the major mutations for improved virulence of SARS-CoV. The resulting proteins 8a and 8b are responsible for caspase-dependent apoptosis and modulating cellular DNA synthesis respectively. [37,38]

Next entry in human coronavirus was Middle East Respiratory Syndrome (MERS-CoV) in the year 2012 and it is also evolved from bats as its ancestral hosts and camels as intermediate hosts. MERS-CoV uses, dipeptidyl peptidase 4 (DPP4) receptors found on human cell surface associated with immune regulation, and signal transduction to infect human. [39] After binding to DPP4, the virus gets activated using a human protease (an enzyme in human cell) triggering the fusion of MERS-CoV virus and human cell. Ancestral host MERS-like bat viruses (HKU4) were unable to infect human, since they could not get activated by human protease after attachment to DPP4 receptor. Bat virus HKU4 and MERS-CoV has two key differences in their genome sequence and this difference only facilitates the human transmission of MERS-CoV. [40]

SARS-CoV-2 is the third zoonotic and pathogenic CoV which has severely affected the human beings after SARS-CoV and MERS-CoV. Genome sequence of SARS-CoV-2 shares around 80% nucleotide identity with the original virus of SARS-CoV. [41,42] However, unlike SARS-CoV, the SARS-CoV-2 has lower mortality and obviously stronger infection ability. [43-45] Since the first lineage was collected in December 2019, there have been many findings on the mutations of SARS-CoV-2 leading to different lineages. During its transmission from body to body (host to host) in last one and half year, it has prospered into genetically diverse tree branching into large number of different lineages. As on 31st July 2021, around 2,553,894 genomes are available in public databases. [46] The other repository NCBI databases have 997404 entries of nucleotide sequence. These repositories get addition of an average of 381 genomes per day from across the globe. [47,48] Most of the reported mutations do not induce significant changes in the biological behaviour of SARS-CoV-2 virus. However, sometimes SARS-CoV-2 genomes coincidently mutates in the “right way” for its survival, replication and transmission. In recent times fast-spreading lineages (B.1.1.7 (alpha), B.1.351 (beta), P.1 (gamma) and B.1.617.2 (delta) lineages) have been reported and off-course they made headlines globally. All of them have resulted from the specific mutations leading to higher transmissibility, infectivity and severity in comparison with the original wild-type (WT) lineage. Commonly, these fast spreading lineages are harbouring mutations which are affecting the interaction of the viral spike receptor-binding domain (S-RBD) with the receptor on host cells, angiotensin-converting enzyme 2 (ACE2). [49,50] Mutations adopted in their genomic sequences have also affected intra-molecular interactions in the receptor-binding domain.

3. Labelling of Mutations in SARS-CoV-2

Generally, a variant/strain/lineage for which there are clear evidence of an increase in transmissibility, more severe disease (e.g., enhanced hospitalizations and deaths), significant reduction in neutralization by generated antibodies either due to previous infection or vaccination, reduced effectiveness for the treatments or vaccines, or detection failures in diagnostic tests are labelled as ‘variants of concern’ (VOCs). Indeed, all the above specified lineages (B.1.1.7 variant, P1 variant, B.1.351 variant, B.1.617 variant) has have been categorized as VOCs. [51,52] Table-1 lists the lineage labelled as VOCs by World Health Organization (WHO) along with their characteristic spike mutations and key outcomes.

Spike protein of SARS-CoV-2 comprises of two independent subunits S1 and S2 that are bonded through non-covalent interactions. Whereas, the S1 subunit contains a receptor-binding domain which mediates the viral entry into the host cells through the binding receptor, the S2 subunit contains two heptad repeat areas that participate in the virus-host cell fusion process. Overall the spike glycoprotein has 1273 residues consisting of five regions, including receptor-binding domain (RBD) (residues 319–541), receptor-binding motif (RBM) that binds to human hACE2 (residues 437–508), fusion peptide (residues 788–806), heptad repeat-1 (residues 920–970) and heptad repeat-2 (residues 1163–1202) as shown in Figure 1. [53]

All the mutation present in VOC given in the Table 1, are primarily NTD and RBD regions of S1 units. It is worth mentioning that RBD is the key region, within which mutations have been found to impact both infectivity and immunity. So far, mutations in the S2 subunit have neither been associated to the virus spread, nor escape from immunity. Since biologically significant mutations of VOC lineages are in the RBD and nearby regions, in the present mini-review focus will be on molecular consequences of biologically key mutations such as L452R, E484K, E484Q, N501Y, K417N, (all in RBD) and D614G in subsequent region. In the following section, manifested molecular consequences of these key mutations and outcome of these consequences in terms of biological functions are discussed one by one.
4. Molecular consequences of key spike mutations in SARS-CoV-2 VOCs

The importance of spike protein is obvious from its surface-exposed location, rendering it a prime target after viral infection for cell-mediated and humoral immune responses as well as artificially designed vaccines and antiviral therapeutics. Therefore, Conformational/structural changes in receptor-binding domain (RBD) of spike protein due to chemical/physical restraints imposed by missense mutation are also of significance. Here are the molecular consequences of each mutation.

4.1. D614G Mutation

In Spike-protein mutation (D614G) aspartic acid is replaced by glycine carboxy (C)-terminal region of S1 domain. Aspartic acid being charged amino acids is important in stabilizing proteins through electrostatic interactions or hydrogen bonds. The acidic moiety of D614 forms a salt bridge with lysine residue (K854) in the fusion peptide–proximal region of S2 unit. Additionally, side chains of D614 protomer (S1-unit) and T859 of the neighboring protomer (S2 unit) form a hydrogen bond in between bringing together S1 domain with S2 (viz. Figure 2).

As expected, substitutions of charged amino acid by non-polar amino acid can alter macromolecular interactions, such as hydrophilic and electrostatic interactions, Van der Waals forces, and hydrogen-bonding networks. The mutation of D614 to G614 (replacing the polar group with non-polar group) eliminates the side-chain hydrogen bonding and salt bridge interaction between S1 and S2 domain. Over all the D614G mutation leads to more flexibility in the S1-S2 binding region (hinge region) because [i] glycine (G) is less bulky than aspartic acid (D) and [ii] loss of hydrogen bond and salt-bridge connections weakens the binding/rigidity. Further it has also been identified that D614G mutation resulted in enhanced affinity of S1–S2 binding region (hinge region) with TMPRSS2 protease due to earned flexibility and accessibility. Interestingly, the protein docking analysis revealed better hydrogen bonding interactions between the Spike protein (S1–S2) cleavage sites (Arg685, Ser686) with catalytic triad of TMPRSS2 in D614G mutant condition as compared to wild-type. For G614 mutant form, Arg682 and residues at primary S1/S2 cleavage site (Arg685 and Ser686) formed six hydrogen bonding interactions with Glu299, Lys300, Asp338, and Gln438 residues of TMPRSS2 (Figures 3), whereas in the D614 wild-type form there were only five hydrogen bonds due to lesser structural flexibility and accessibility. The binding energy was observed to be better for the G614 mutant (−143.03 kcal/mol) as compared to that of the wild type (−113.67 kcal/mol), indicating better binding of TMPRSS2 with the mutated Spike protein.

Overall, D614G mutation induced enhanced affinity of S1–S2 cleavage site towards TMPRSS2 protease, which, results in increased shedding of S1 domain (dipping of S1 domain on hACE2) in G614 as compared to D614 concomitant with increased infectivity of the D614G mutant.

4.2. N501Y Mutation

Cryo-electron microscopy (cryo-EM) and X-ray crystallography structural studies have identified N501 as a key residue in spike protein at the interface between receptor binding domains (RBDs) and hACE2 that is involved in key contacts with several hACE2 residues. In N501Y mutations, asparagine (N) is being replaced with tyrosine (Y) at position 501 of the RBD (viz. Figure 4). Replacement of asparagine with tyrosine does not lead to any significant changes in secondary or quaternary structure. Even though this mutation does not alter the structure features of isolated RBD, but significant modification has been observed for the RBD-hACE2 complex. In the RBD-
hACE2 complex, hydrophilic N501 remain in the proximity of the hydrophobic benzene ring of Y41 residue and the hydrophobic alkane chain in K353 residue of hACE2. Therefore, it is expected that the interfacial interactions will be strengthened when a hydrophilic asparagine is being substituted by a hydrophobic residue tyrosine having an aromatic ring. The

| Lineage   | Country first detected | Characteristic Spike protein mutations and (biologically significant are underlined) | Key Attributes (compiled from WHO report) |
|-----------|------------------------|----------------------------------------------------------------------------------------|------------------------------------------|
| B.1.1.7   | United Kingdom         | 69/70del, 144del, N501Y, A570D, D614G, P681H, T716I, S982A, D1118H                   | Increased transmissibility                |
|           |                        |                                                                                        | Possible increased risk of hospitalization, severity and mortality |
|           |                        |                                                                                        | Neutralizing activity retained           |
|           |                        |                                                                                        | No/Minimal impact on some of the vaccine performance |
| B.1.351   | South Africa           | D80 A, D215G, 241/243del, K417N, E484K, N501Y, D614G, A701V                           | Increased transmissibility               |
|           |                        |                                                                                        | Possible increased risk of in-hospital mortality |
|           |                        |                                                                                        | Reduction in neutralizing activity       |
| B.1.1.28.1 | Brazil, alias P.1      | L18F, T20N, P26S, D138Y, R190S, K417T, E484K, N501Y, D614G, H655Y, T1027I, V1176F | Increased transmissibility               |
|           |                        |                                                                                        | Possible increased risk of hospitalization |
|           |                        |                                                                                        | Moderate reduction in neutralizing activity reported |
|           |                        |                                                                                        | Minimal/moderate impact on some of the vaccine’s performance |
| B.1.617.2 | India                  | T19R, G142D, L452B, T478K, D614G, P681B, D950N, del157/158                          | Increased transmissibility               |
|           |                        |                                                                                        | Possible modest reduction in neutralization activity |
|           |                        |                                                                                        | Minimal/moderate impact on some of the vaccine’s performance (under investigation) |

Figure 2. (a) Schematic representation of Chemical nature of residue amino acids in D614G mutation. (b) Interaction of D614 residue of S1 unit with S2 unit (PDB-ID: 6VSB) (c) Interaction of G614 residue of S1 unit with S2 unit (PDB-ID: 6SX6) Part (b) and (c) of image has been formulated at RCSB website http://www.rcsb.org/structure/using data available in Protein Data Bank
aromatic ring of Y501 gets sandwiched between Y41 and K353 of the hACE2 receptor and consequently the benzene ring edge forms the perpendicular π–π stacking interaction with the benzene ring surface of Y41, and simultaneously Y501 benzene ring surface also interacts hydrophobically with the alkane chain in K353 as shown in Figure 4(b). Overall, the N501Y mutation induces few additional associations with hACE2: (i) potentially π−π stacking interaction with Y41 residue and (ii) a cation−π interaction with K353 residue (iii) a hydrogen bond with D38 residue (iv) increased salt-bridge electrostatic interaction between T500 and D355 in the RBD and hACE2 respectively. [65,66] These new associations results in an increase in hACE2-binding affinity which furthers effects the infectivity. [67] These inferences are in line with a recent report demonstrating increased cell entry of pseudoviral particles incorporating both N501Y and D614G mutations in comparison to pseudoviral particle harbouring D614G mutation alone. [68] 4.3. E484K Mutation E484 is a site where mutations are present in several SARS-CoV-2 VOCs. The 484th residue position is in Receptor Binding Motif (RBM) of the RBD existing in S1 subunit and mutation at this position is expected to affect binding and neutralization potency. [70] Glutamate occupies this 484th position (E484) located at the edge of RBD-hACE-2 interface (viz.Figure 5). In E484K mutation, negatively charged glutamic amino acid is replaced by positively charged lysine amino acid. [71] By analysing the RBD-hACE2 binding interface, it is found that around the glutamate 484 (E484), there exist several charged residues on the receptor hACE2, including Glu35, Asp38 and Glu75 with negative charges, along with Lys31 with positive charge (viz.Figure 5). On average, the interface side of hACE2 exposed to RBD mainly has electronegative character, which is unfavourable for electrostatic interactions with negatively charged Glu484. Conversely, replacement of glutamic acid (E) by lysine (K), these unfavourable gas-phase electrostatic interactions involving the residue 484 were converted in to favourable interactions and changes the dynamics and properties of virus S protein [60]. Since this 484th
position exist in a highly flexible loop, it has been found that E484K mutation also causes conformational rearrangements of the local structure in the surrounding (shift of loop) as shown in Figure 5(c).

Moreover, during RBD-hACE2 binding this loop shift leads to formation of additional hydrogen bonding interactions in the compatible residues. This more favourable electrostatic interactions and formation of new hydrogen bonds due to E484K mutations, provides additional stability to the RBD-hACE2 binding. The aforementioned improved affinity is a likely culprit for more rapid spread of this variant due to greater transmissibility, which is a prime reason why it is important to track these mutations and act in a timely manner.

During the interaction of RBD with neutralizing antibody, the E484 residue is involved in large number of hydrogen-bonds and salt-bridge interactions. Figure 6 shows these interactions between E484 and neutralizing antibodies namely BD368-2, P5A-1B9, P2B-2F6 and CV07-270 monoclonal antibodies (mAbs). E484K mutation disrupted these H-bonding and salt-bridge interaction resulting in reduced effectiveness of these antibodies. More importantly, the K417N mutation allows the variant to escape from many human antibodies by removing a salt-bridge buried in the RBD-antibody interface.

4.4. K417N Mutation
In K417N Spike-protein mutation charged lysine is replaced by neutral asparagine. In the SARS-CoV-2 wildtype RBD, K417 forms a salt bridge with the D30 residue in hACE2 and is important in RBD-hACE2 recognition (viz. Figure 7). Thus, the K417N mutation results in the abolishment of this favourable interfacial interaction and reducing the binding affinity between the RBD and hACE2. The isolated K417N mutation has been reported to decrease the hACE2 binding by about 6.4-fold.

Figure 8 shows the various salt-bridge interaction of K417 residue with various antibodies. K417 in RBD can form a buried salt-bridge with either a glutamate or an aspartate in four different human antibodies. More importantly, the K417N mutation allows the variant to escape from many human antibodies by removing a salt-bridge buried in the RBD-antibody interface. Few reports suggest that K417N mutation is less disruptive to P2 C-1F11 neutralizing antibodies in comparison to other antibodies because P2 C-1F11 binds to the wildtype (WT) K417 through hydrophobic and hydrogen-bond interactions. Thus, disruption of salt bridge by mutation
Binquan Luan and co-workers claimed that K417N mutation seems to sacrifice its binding affinity for hACE2 in order to survive the attack of antibodies.

4.5. E484Q Mutation

As discussed in previous section-4.2, 484th residue is a key position located at the edge of RBD-hACE-2 interface and glutamate occupies this position. So far, the influence of mutation E484Q has been reported as co-mutant along with L452R. Substitution of charged glutamate with neutral glutamine disrupts an electrostatic bond of the spike RBD residue E484 with K31 in the hACE2 interaction interface as shown in Figure 9. E484Q have been reported to disrupt the interfacial interactions of spike RBD with specific neutralizing antibodies. Monoclonal antibody ‘REGN10933’ interacts with the RBD by making two hydrogen bonds (H-bonds) between E484 of the RBD and Y53 and S56 of the antibody. The RBD mutation E484Q disrupts these two H-bonds with S56 and Y53.
4.6. L452R Mutation

L452 residue is part of the 443–450 loop located on the edge of the receptor-binding motif (RBM) of RBD. The L452 residue does not form direct contact with the receptor, but it is part of hydrophobic together along with F490 and L492 as shown in Figure 10. The L452R mutation, in which hydrophobic leucine is replaced by hydrophilic arginine is seen to abolish these hydrophobic interactions, likely because of its greater affinity towards water molecules after mutations. Furthermore, the mutation also induces local conformational changes. These confirmation changes in the RBD promote the interaction between the spike protein and its hACE2 receptor.  

Even though the L452 residue is not directly located at RBM-hACE2 interface, this mutation increases electrostatic interactions with hACE2. Figure 10 shows the close proximity of 452nd residue with negatively charged patch of hACE2 residues (E35, E37, D38). Hence, substitution of neutral leucine with charged arginine at 452nd position leads to increase in the electrostatic interaction and hence enhanced binding with hACE2 which in turn may significantly increase the viral transmissibility and infectivity.  

L452R mutation also reduces the virus-neutralizing ability of antibodies. L452R induced conformational changes affects binding of viral S protein with neutralizing antibody binding. For example inter-molecular hydrophobic interactions of L452 with I103 and V105 residues of heavy chain of P2B-2F6 antibody break due to L452R mutation. Overall, replacement of leucine with arginine at 452nd position results in both a much stronger binding to the receptor and escape from neutralizing antibodies.

4.7. P681H & P681R mutations

P681 residue site is adjacent to S1-S2 Furin cleavage site (FCS) having binding sequence Arg-Arg-Ala-Arg (RRAR) of Spike protein as shown in Figure 11. The multiple arginine residues make this stretch as poly-basic (multi-basic). When hACE-2 receptor binds with spike protein, it induces conformational changes in the region of S1–S2 FCS mediating the subsequent cleavage and viral fusion with target host cell. Thus mutation at P681 site becomes important for secondary structure character in this region. It has been found that replacement of proline (P) with Histidine (H) at 681st residual position (P681H) increase molecular flexibility (enhanced coil preference as shown in Figure 7).
Figure 12) which in turn increases the accessibility of Furin for binding at cleavage site. Moreover, there is an increased contact between H681 and R683 residue after the mutation (viz. Figure 13), which leads to the extra stabilization of canonical FCS and in turn assists in more efficient recognition and cleavage finally resulting in better transmissibility.\[73,89,90,91\]

The P681H mutation also results in a slight enhancement of polar character (due to Histidine) which in turn increases the stability in aqueous environments, and alters the neutralizing antibody binding properties.\[73\]

In another case when proline (P) at 681\textsuperscript{th} residual position is replaced with arginine (R) (P681R), results in further enhancement of the basicity of the poly-basic stretch (RRAR), which might help in increased rate of membrane fusion, internalization and thus better transmissibility. The convergent role of L452R, E484Q and P681R in virus infectivity has also been reported previously.\[82\]

4.8. T478K Mutation

478\textsuperscript{th} residue position is part of important T470-T478 loop present at the RBD-hACE2 interface (viz. Figure 13).\[92\] T478K mutation leads to replacement of amino acid from the polar and uncharged threonine (T) to positively charged lysine (K). This mutation changes the electrostatic surface of spike protein making its surface more positive and increases its electrostatic potential. The electrostatic surface gets further modified by co-occurring mutations.\[93\] Muecksch and co-workers through their experimental investigation showed that T478K and T478R mutations are the possible genetic route for escaping immune recognition.\[94\]

5. Synopsis of different VOC lineages

In this section synopsis of spike mutations at a glance for the different Variants of Concerns (VOCs) of SARS-CoV-2 will be depicted.

5.1. B.1.1.7 Lineage (alpha variant)

The SARS-CoV-2 lineage B.1.1.7 was first detected in late 2020. This lineage spread rapidly across England between November 2020 and January 2021.\[95\] A large number of non-synonymous
substitutions of immunological importance have been observed in this lineage. It is defined by 23 mutations from the original wild type virus (Wuhan Strain), 8 of which are in the spike protein. N501Y, D614G and P681H are the mutations of peak biological significance present in this lineage (viz. Figure 14). It has been suggested that this lineage is more transmissible and causes higher chance of hospitalization, though it does not appear to lessen vaccine efficacy.[50, 51] Biomarker (D69-70) Deletion was associated with diagnostic test failure for a probe targeting the spike gene (FDA, 2020).[95, 96]

![Diagram showing chemical nature of residue amino acids in L452R mutation, hydrophobic stretch around L452 residue, location of L452 residue of S1 RBD during complexation with hACE2 unit, and interactions of L452 residue with monoclonal antibody P2B-2F6.](image_url)

Figure 10. (a) Schematic representation of Chemical nature of residue amino acids in L452R mutation. (b) Hydrophobic stretch around L452 residue. (c) Location of L452 residue of S1 RBD during complexation with hACE2 unit (PDB-ID: 6LZG). (d) L452R mutation induced enhanced electrostatic interaction and hence enhanced binding with hACE2 (e) Interaction of L452 residue with monoclonal antibody P2B-2F6 (PDB-ID: 7BWJ). Part (b, c and e) of image has been formulated at RCSB website http://www.rcsb.org/structure/using data available in Protein Data Bank. Part (d) of image is reproduced from ref-84 open access article under the CC BY license bioRxiv preprint https://doi.org/10.1101/2021.04.02.438288 © 2021 The Authors/funder. Published by bioRxiv.
5.2. B.1.351 Lineage (Beta variant)

B.1.351 lineage is also known as 501Y.V2. This lineage also has the same type of N501Y mutation as found in the B.1.1.7 lineage but it contains additional key mutations such as K417N, and escaping E484K mutation (viz. Figure 15). It has been reported that the combination of E484K, K417N and N501Y results in the highest degree of conformational alterations during RBD hACE2 binding in comparison to isolated mutation either E484K or N501Y. Zhou and co-worker showed that the escape of this lineage from monoclonal antibody neutralization is largely driven by E484K, although K417N and N501Y act together against some important antibody classes. The above mentioned features result in increased transmission, potential increased severity and moderate effect on neutralization activity and vaccine’s efficacy.

Figure 11. (a) Schematic representation of Chemical nature of residue amino acids in P681H and P681R mutation. (b) Location of P681 residue adjacent to S1-S2 Furin cleavage site (FCS) having binding sequence Arg-Arg-Ala-Arg (RRAR). Part (b) of image is reproduced with permission from ref-87 Science 2020, 370, 861–865 (DOI: 10.1126/science.abd3072) Copyright 2020 American Association for the Advancement of Science.

Figure 12. Increasing molecular flexibility by P681H as few bonds are disrupted. Image is formulated using COVID-3D tool at http://biosig.unimelb.edu.au/covid3d/mutation/QHD43416/ACE2_BOAT/P681H/A

Figure 13. (a) Schematic representation of Chemical nature of residue amino acids in T478 K mutation. (b) Location of T478 residue of S1 RBD during complexation with hACE2 unit (PDB-ID: 6LZG). (c) T478 K mutation induced enhanced electrostatic interaction and hence enhanced binding with hACE2. Part (b) of image has been formulated at RCSB website http://www.rcsb.org/structure/using data available in Protein Data Bank. Part (c) of image is reproduced from ref-93 open access article under the CC BY license bioRxiv preprint doi: https://doi.org/10.1101/2021.03.28.437369 © 2021 The Authors/funder. Published by bioRxiv.
5.3. P.1 Lineage (Gamma variant)

This lineage is a close relative of the B.1.351 lineage. The P.1 lineage of SARS-CoV-2 likely arose in Brazil, during mid-November 2020.\(^9\) This lineage also has characteristic triple mutation (K417T, E484K, N501Y) in spike protein (viz. Figure 16). The same three residues are mutated with in the B.1.351 variant of concern, and N501Y is also present in the B.1.1.7 lineage. K417T indicates that lysine at position 417 was replaced by threonine and its consequences are expected be similar as that of K417N.\(^1\) Overall epidemiology of this lineage is quite similar to that of beta variant.

5.4. B.1.617.2 Lineage (Delta variant)

The B.1.617 variant was first detected in India and was divided in three lineages - B.1.617.1, B.1.617.2 and B.1.617.3. Although it possesses 12 mutations in its spike protein relative to the wildtype SARS-CoV-2, B.1.617.2 lacks mutations at amino acid positions 501 or 484 in its hACE2 receptor-binding domain (viz. Figure 17), commonly associated with VOCs or escape from neutralizing antibodies (NAbs).\(^1\) B.1.617 possessing common signature mutations L452R, E484Q, D614G and P681R, in the spike protein but the B.1.617.2 lineage does not possess E484Q mutation. Detailed structural analysis by Cherian and co-workers reveals that mutations L452R and E484Q along with P681R in the Furin cleavage site may leads to increased hACE2 binding and enhanced rate of S1–S2 cleavage resulting in better transmissibility.\(^6\) While L452R mutation has been associated with reduced antibody efficacy and reduced neutralization by vaccine sera, other key mutation P681R has been associated with chemical processes that may enhance transmissibility.\(^6,8\) However it is still under investigation that how quickly B.1.617 variants can spread, and whether they can evade immunity.\(^10\)

6. Overview and perspective

In the above sections the picture at the molecular level interaction (intra-molecular-within spike protein and inter-molecular i.e. between spike protein and human cell receptor/
antibodies) has been portrayed. From the discussion, it is clear that even changing a single nucleotide (in turn single change in amino acid sequence-missense mutation) can significantly change the chemical characteristics and if the region of bearing changes is a biologically important region, then it will lead to significant changes in the biological functions. For SARS-CoV-2 lineages, mutations occurring in and around the two regions (i) receptor binding motif (RBM) region and (ii) S1/S2 Furin cleavage site (FCS) region have significant biological consequences i.e. enhanced infectivity, neutralization by antibodies etc. Opening the structure of spike protein owing to mutation induced molecular flexibility (disruption/weakening of intra-molecular interaction to remove the rigidity within the spike protein as observed case of E484K or L452R) allows the exposure of spike protein surface to human receptor. For P681H or D614G mutation induced molecular flexibility facilitates the Furin accessibility for S1/S2 cleavage. In N501Y case mutation results in significant strengthening of inter-molecular interactions between RBD and hACE-2 due to addition of hydrogen bonds, salt bridge etc.

Neutralization of virus by antibody is significantly dependant on the binding or inter-molecular interactions between spike antigen (or epitope-binding region of antigen) and antibody. If mutation in the epitope region is hampering these inter-molecular interactions, it will reduce the neutralization efficacy of antibody. Indeed this was observed for the mutation occurring at E484, where replacement of glutamate results in disruption of large number of hydrogen-bonds, salt-bridge interactions with various monoclonal antibodies. Due to this reason E484K have potential to impact the neutralization by antibodies and is known as escape mutant to evade immune response.

Most neutralizing antibodies to SARS-CoV-2, block the viral entry to cell by binding to epitope of spike antigen and not allowing the host cell ACE-2 receptor to interact with RBD. Antibodies(AB) compete with host-cell ACE2 for binding with spike protein-RBD and if they are successful in this rivalry, they will stop the ACE2 to bind with RBD and in turn disrupt the cell infection. Obviously, the race of antibody and hACE2 receptor for antigen spike binding is dictated by dynamics of competing molecular level interactions. The mutation induced change in the molecular interaction have direct impact on this completion and hence on neutralization efficiency. E484K and K417N mutations weaken the antibody-spike antigen interaction which results in reduced sensitivity to neutralizing antibodies. Without the key information about competing AB-RBD and hACE2-RBD molecular interactions, it is difficult to develop vaccines that can induce protective and durable immunity. A precise knowledge of these competing chemical affinities and their dynamics in physiological conditions (stability, conformational changes, hydrogen bonds, salt bridges, Van-der-Waals interactions and hydrophilic interactions, secondary and tertiary structures) will help in designing of more potent neutralizing antibodies.

Further, it is also clear that human to human transmission pattern (Contact and droplet transmission via respiration) is same for wild type and mutated virus and mutation has not induced any new transmission mode. Thus, to combat the pandemic with emerging new variants, same old procedures (facial coverings, social distancing and practicing proper hygiene and sanitation) are still the key pillars.

7. Summary
Recently, mutations in the spike protein of SARS-CoV-2 have been of great concern due to their potential for enhanced transmissibility and immune escape. Different deletion and substitution in spike protein causes significant alteration in its structure, stability and molecular surface character leading to evolution of different variants of SARS-CoV-2. WHO has labelled few viral particle as of “variant of concern” (VOC) when they shows evidence of fulfilling few criteria, such as easy transmission, more illness severity, reduced antibody neutralization or reduced effectiveness of treatment and vaccines. As on 20th July 2021, four lineages Alpha (B.1.1.7), Beta (B.1.351), Gamma (P.1) and Delta (B.1.617.2) have been kept under the category of VOCs. The mutation induced modified epidemiology of these SARS-CoV-2 lineages needs to be contextualized with their molecular structure to enable a suitable and prompt response against them. With an aim to rationalize mutation’s impact on virus biology, in this review, we explore the literature on key spike mutations harboured by these SARS-CoV-2 VOCs. Focus of the article has been on portraying the molecular picture of critical spike mutations of these four SARS-CoV-2 lineages (B.1.1.7, B.1.351, P.1 and B.1.617.2) in simplified manner and correlates it with their observed biological response.

Usually, during the mutation process chemical nature of substituting amino acid modifies the involved interactions like hydrogen bonds, Van-der-Waals bonds, salt bridges, hydrophobic interactions etc. which in turn changes the biological functions of virus. Replacement of polar residue with non-polar, charged with uncharged, positive charge with negative one, or larger residue with smaller one noticeably influenced/hampers the chemical interactions. The hampered interaction can be intra-molecular (for e.g. within the polypeptide chain) and/or inter-molecular (for e.g. interaction between polypeptide chains). Present review has detailed the molecular consequences of critical mutations observed at residue position 417, 452, 484, 501, 614 and 681 in the spike protein of SARS-CoV-2. The lineages labelled as VOCs by WHO bears (B.1.1.7, B.1.351, P.1 and B.1.617.2) one or more mutation among these. D614G mutation enhances the affinity of S1/S2 Furin cleavage site towards TMPRSS2 protease allowing better accessibility of S1-RBD for hACE2. Other two mutations N501Y and E484K allow additional inter-molecular interaction between S1-RBD and hACE2 leading to increased cell entry and infection. E484K and K417N mutation abolish many interfacial interactions between spike antigen and neutralizing antibody which assists in escaping from immune response. In L452R mutation, change from non-polar to polar amino acid residue enhances the electrostatic affinity of S1-RBD and hACE2. Similarly substitution of non-polar amino acid by polar one at P681 residual site affects the secondary structure of poly-basic S1-S2 Furin cleavage site (FCS). Commonly for SARS-CoV-2 VOC lineages, mutation
occurring in and around two regions (ii) receptor binding motif (RBM) region and (ii) S1/S2 Furin cleavage site (FCS) region have significant biological consequences. Mutation induced disruption of intra-molecular interaction within spike leading to enhanced flexibility (open conformation) and approachability has been one of the salient observations in the explored lineages. Competing inter-molecular interactions Ab-RBD and hACE2-RBD gets decisive influence by mutations which dictates the antigenicity and mediated immune response.

Overall, molecular exploration of different VOCs of SARS-CoV2, presented in the article with integrative chemical biology yet simplified approach, is expected to offer key learning point across different discipline. It is believed that consolidation of crucial information regarding molecular basis of SARS-CoV-2 spike mutations of VOCs at single place will help not only in expediting the coherent interdisciplinary future efforts rather it can help in creating a common consensus for better management of severity load enabled by these VOCs.

Author Contributions
NJ and AT were responsible for literature search and collection of data and formulating the figures. AT and SN were responsible for concretizing and drafting the article and interpreting the data. SN was responsible for the sequencing of content flow, interfacing the content at boundary of chemistry and biology, revising the article for important intellectual content flow, interfacing the content at boundary of chemistry and biology, revising the article for important intellectual content. All authors were also involved in discussions at various stages. All authors have given approval to the final version of the manuscript.

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Conflict of Interest
The authors declare no conflict of interest.

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