Research Article

Journal of Clinical Rheumatology Research

Mutational Spectra of Sars-Cov-2 And Ace 2 Can Modify Infectivity, Immunogenicity and Disease Severity: A Review

Sarmishtha Chanda*

Sister Nibedita Govt. General. Degree College for Girls. Hastings House. 20 B Judges Court Road, Alipur, Kolkata 700027.

***Corresponding author**

Sarmishtha Chanda, Sister Nibedita Govt. General. Degree College for Girls. Hastings House. 20 B Judges Court Road, Alipur, Kolkata 700027.

Submitted: 23 Apr 2022; **Accepted:** 30 Apr 2022; **Published:** 05 May 2022

Citation: *Sarmishtha Chanda. (2022). Mutational Spectra of Sars-Cov-2 And Ace 2 Can Modify Infectivity, Immunogenicity and Disease Severity: A Review, J Clin Rheum Res, 2(1),30-39.*

Abstract

COVID 19 is primarily characterized as severe respiratory syndrome, careful review revealed that it has multisystem involvement (Mao et al 2020). Due to its potentially damaging effects on respiratory system it is termed as SARS- COV 2 (severe acute respiratory syndrome corona virus 2). However, severity of the disease differentially changes from one to another geographical area and on the viral load of people affected. It has been shown from recent studies that the virus acquired several mutations on the spike protein which is the receptor binding domain of the virus particle. These mutations also alter the glycosylation pattern of the viral envelop. Mutation also differentially glycosylate the S1 and S1 domain of the spike protein and thereby modulate its infectivity through changes the potentiality of binding with ACE2 receptor. Changes in infectivity may also alter immunogenicity and manifestation spectra of the disease. ACE 2 receptor mutation site also perturb the glycosylation pattern and therefore alters the binding capacity with virus. All these mutations may potentially alter disease severity which is reported worldwide. Mutation of SARS-CoV 2 and ACE2 receptor in combination or in alone therefore modulate the entire picture of disease scenario which is the most interested part of COVID research. In this review we are going to unrevealing these areas of COVID -19 disease spectra.

Keywords: Mutation, S1 and S2 domain, Spike protein, ACE2 receptor, glycosylation, binding, disease severity

Introduction

In December 2019, Corona Virus Disease 2019 (COVID-19) epidemic emerged in Wuhan, China, causing global threat (Thompson, 2020) when a series of patients presented with fever and pneumonia of unknown etiology. COVID-19 is primarily characterized by respiratory system involvement, causing severe respiratory illness. It has spread rapidly worldwide and on March 11, 2020, the World Health Organization proclaimed this disease as a pandemic. Although COVID-19 is primarily characterized as severe respiratory distress syndrome, careful review revealed that it has multi-system involvement. Because of its potentially damaging effects on the respiratory system, it is termed as SARS-CoV-2 (severe acute respiratory syndrome coronavirus 2). Most patients suffering from COVID-19 have mild to moderate respiratory symptoms. Although careful judgment reveals that SARS-CoV-2 involves multiple systems and could lead to multiple organ failure, lungs are usually the most severely affected. The etiology of COVID-19 shows that it causes mild to moderate and even severe manifestations in people belonging to various age and sex categories. People with similar underlying pathological conditions like hyperglycemia or hypertension can even manifest in different extents after SARS-CoV-2 infection. We hypothesize that this differential degree of clinical manifestations lies within the factors controlling the disease pathology and immunology, and in the genetic predisposition of individuals. Polymorphism of genes directly involved in the morphological or structural specification of the virus at its cognitive domain, which is specially designed to bind with specific receptor on the host cells, are most relevant in the diverse manifestation of SARS-CoV-2 induced illness.

In the last 20 years, researchers have isolated seven different corona viruses responsible for respiratory syndromes of varying severity in humans. Most of them, including SARS-CoV-2, are from beta coronavirus lineage, and can cause lung injury, including multi organ failure, in severe cases. SARS-CoV-2 is the ligand of human angiotensin I converting enzyme 2 (ACE2) receptor. Although ACE2 receptors are predominantly present in the lung alveolar cell membrane, ACE2 receptor mRNAs are present virtually in

all organs including heart, blood vessels, kidney and testis, thus opening possibilities of the virus to bind to the other tissues and infect them. The ACE2 molecule is a peptidase which regulates the water and salt balance of the body by binding with angiotensin II, through renin-angiotensin-aldosterone system, thus acting as the main regulatory pathway of controlling blood pressure and cardiac output. This implies that underlying pathology like hypertension, diabetes and cardiomyopathy of a person are major contributing factors to develop severe manifestations of SARS-CoV-2 infection.

Although ACE2 receptors are predominantly present in the lung alveolar cell membrane, ACE2 receptor mRNAs are present virtually in all organs including heart, blood vessels, kidney and testis, thus opening possibilities of the virus to bind to the other tissues and infect them. The ACE2 molecule is a peptidase which regulates the water and salt balance of the body by binding with angiotensin II, through renin-angiotensin-aldosterone system, thus acting as the main regulatory pathway of controlling blood pressure and cardiac output. This implies that underlying pathology like hypertension, diabetes and cardiomyopathy of a person are major contributing factors to develop severe manifestations of SARS-CoV2 infection.

Although ACE2 receptors are predominantly present in the lung alveolar cell membrane, ACE2 receptor mRNAs are present virtually in all organs including heart, blood vessels, kidney and testis, thus opening possibilities of the virus to bind to the other tissues and infect them. The ACE2 molecule is a peptidase which regulates the water and salt balance of the body by binding with angiotensin II, through renin-angiotensin-aldosterone system thus acting as the main regulatory pathway of controlling blood pressure and cardiac output. This implies that underlying pathology like hypertension, diabetes and cardiomyopathy of a person are major contributing factors to develop severe manifestations of SARS-CoV2 infection.

Although ACE2 receptors are predominantly present in the lung alveolar cell membrane, ACE2 receptor mRNAs are present virtually in all organs including heart, blood vessels, kidney and testis, thus opening possibilities of the virus to bind to the other tissues and infect them. ACE2 molecule is a peptidase which regulates the water and salt balance of the body by binding with angiotensin II, through renin-angiotensin-aldosterone system thus acting as the main regulatory pathway of controlling blood pressure and cardiac output. This implies that underlying pathology like hypertension, diabetes and cardiomyopathy of a person are major contributing factors to develop severe manifestations of SARS-CoV2 infection.

Although ACE2 receptors are predominantly present in the lung alveolar cell membrane. ACE2 receptor mRNAs are present virtually in all organs including heart, blood vessels, kidney and testis, thus opening possibilities of the virus to bind to the other tissues and infect them. ACE2 molecule is a peptidase which regulates the water and salt balance of the body by binding with angiotensin II, through renin-angiotensin-aldosterone system thus acting as the main regulatory pathway of controlling blood pressure and cardiac output. This implies that underlying pathology like hypertension, diabetes and cardiomyopathy of a person are major contributing factors to develop severe manifestations of SARS-CoV2 infection.

The virus, an encapsulated ssRNA particle, also shows divergent strains with varying mutations in their RNA strand and the envelope protein. These mutations handle differential binding ability with the host cell and causes either facilitation or inhibition for the entry of viral particle. The binding of the viral particle with the ACE2 receptors occurs through viral spike protein (S). This S is the dimer of S1 and S2. Wet lab study and bioinformatics showed that about 4-5 different SNPs are present within the S1 and S2 domain of the S protein receptor binding domain (RBD), which may alter or perturb the functional attachment of the S protein with ACE2 receptor. Thus, a detailed understanding of SARS-CoV-2 Spike binding to ACE2 is at the Centre of interest to explain not only the mechanism of viral binding and entry to the host cell, but also for the effective therapeutic understanding. To assess the genetic variations of different SARS-CoV strains, National Centre for Bioinformatics, China, has worked out over 77,801 genome sequences of SARS-CoV-2 from all over the world. They identified a total 15,018 mutations, including 14,824 SNP. All these mutations may or may not interact directly to the binding potency of the virus and may therefore affect the extent of manifestations in individuals.

It has been found that each individual has a distinct expression profile for the ACE and ACE2 receptors. This implies varying degrees of expression of the ACE/ACE1 and ACE2 receptors, which may significantly alter the disease profile of the virus. Renin-Aldosterone-Angiotensin system is important in maintaining water balance and blood pressure regulation within the body and, therefore, plays a major role in the pathogenesis of COVID-19. The angiotensin converting enzyme (ACE or ACE1) catalysis the synthesis of Angiotensin II, which is the main regulator for increasing blood pressure, whereas ACE2 hydrolyses ACE-II to Ang 1-7 [1,2]. When Angiotensin binds to angiotensin receptors (AT1) it causes vasoconstrictions and increase in peripheral blood pressure, but on the contrary, when Ang 1-7 binds to angiotensin 2 (AT2) it produces vasodilation. Therefore, ACE and ACE2 act antagonistically to balance the blood pressure and cardiovascular events, relating the reduced expression of ACE2 to the development of cardiovascular diseases.

It is seen from various studies that hypertension and cardiovascular disease are significant comorbidities in COVID-19, and are increasingly associated with stroke and CoVID induced encephalopathy and eventual death in elderly as well as young patients. Although ACE2 receptors predominantly present on alveolar cell membrane, its widespread distribution leads to a varying degree

and diversified symptoms of COVID-19 [3]. Besides respiratory complications, the most common outcomes of COVID-19 are acute cardiac injury and acute kidney injury. Rectal swab, saliva, urine samples from CoVID positive patients shows enough viral load to detect by RT-PCR. These findings clearly demonstrate that SARS-CoV-2 is distributed widely in an infected body, with a distribution pattern is similar to that of ACE2 [3]. An analysis on the single cell RNA sequencing data from human organs revealed that cells showing expression of ACE2 included 7.5% of cardio myocyte, 4% of proximal tubular epithelial cell, 2.4% of bladder urothelial cell, 30% ileum epithelium cell, 1% esophageal epithelial cell and 2% of respiratory tract epithelial cell [4]. These wide ranges of tissue distribution of ACE2 receptor are the primary reason for the wide range of clinical manifestations in SARS-CoV-2 infection and multiorgan failure in severe infection.

Ace Gene and Its Expression

ACE 1 gene is found on Chromosome 17. The genomic location is 17q23.3. ACE inhibitors are commonly used to treat hypertension and some types of renal and cardiac dysfunction. It converts angiotensin I to angiotensin II by release of the terminal His-Leu, to increase the vasoconstrictor activity of angiotensin. ACE1 can also inactivate bradykinin, a potent vasodilator and has a glycosidase activity which releases GPI-anchored proteins from the membrane by cleaving the mannose linkage in the GPI moiety. ACE1 is strongly activated by chloride.

The ACE2 gene is located on chromosome X. The ACE2 gene maps onto chromosome Xp22. The gene, which has 18 exons, encodes an 805 amino acid metalloproteinase, with the functional protein existing as a type I integral membrane glycoprotein containing a single catalytic domain [3]. Altered ACE2 expression is associated with cardiac and vascular disease in experimental models of CVD, and increased levels of ACE2 have been implicated in human heart failure and atherosclerosis. The location in X chromosome is a disadvantage for mail carriers of alleles linked to a lower ACE2 expression and therefore higher prevalence of COVID-19 severity in males [5]. Circulating ACE2 activity increases with CVD, as well as heart failure, and a large proportion of the variation in plasma ACE2 levels has been attributed to hereditary factors or which was unexplained since last decade, the polymorphic variation with in the gene. SARS-CoV down-regulates the circulating level of ACE2 level might be through down regulating and therefore take an advantage to promote damage and inflammation to lung and heart. Actually, ACE1 and ACE2 work in a balanced way but in opposite direction in the renin–angiotensin system (RAS) to balance the local vasoconstrictor/proliferative (ACE1/Ang-II/AT1-axis) and vasodilator/ant proliferative (ACE2/Ang1-7/MAS-axis) actions to combat dysregulated blood pressure, water and mineral balance [6]. This protects organs and blood vessels by anticoagulant, anti-inflammatory, anti-proliferative, anti-fibrosis, anti-alveolar epithelial cell apoptosis, and anti-oxidative stress activities antagonizing the effect of Angiotensin II. It is therefore worthy that, the coexistence of gene polymorphisms both in the ACE1 and ACE2 genes might influence their mutual expression levels which might be associated with altered salt balance or blood pressure regulation along with CVD, increased capillary permeability, coagulation, fibrosis, and apoptosis in the alveolar cells, accelerating lung damage and pulmonary shut-down triggered/worsened because of SARS-CoV-2 infection [7]. Therefore, careful evaluation must be conducted for ACE2 gene polymorphism and infection outcomes with relative severity in different population with different age and sex matched individuals. In fact, ACE gene insertion/deletion (I/D) is one of the best known polymorphisms which affects the circulating level of ACE and ACE2. For example, an individual with a D/D genotype shows higher levels of ACE2 in the circulation, which can explain higher incidence of SARS-CoV-2 infection in those individuals and with higher risk of cardiovascular and respiratory disease with homozygous deletion genotype. This homozygous deletion is further related to acute respiratory distress syndrome (ARDS) with a concomitant progression of pneumonia [8]. As ACE2 is the receptor for SARS-CoV-2 attachment, ACE2 polymorphism or functional variants of ACE2 receptor could practically limit the entry of SARS-CoV-2 on the cell surface and facilitate or inhibit the severity of the disease. The functional variants that increase the expression of ACE2 receptor protein could cause increased viral load by displaying increased number of viral binding sites and therefore increase the vulnerability of carriers to infections. These risk variants are particularly higher in male as they possess only one copy of X linked ACE2 gene. ACE I/D can exist in three different polymorphic forms, DD, ID, and II. The occurrence of severe COVID-19 disease is associated with DD or ID genotype whereas in case of II genotype the occurrence is quite low, although the association of ACE2 I/D polymorphism with the disease progression has not been found [8].

The catalytic domains of ACE2 and ACE1 share about 42% homology in their amino acid sequence and have a similar exon/intron organization. Although several gene variants of ACE2 have been identified, one SNP, i.e. transition G8790A (rs2285666) in intron 3 of the ACE2 gene, is of special interest. Unlike autosomal genes (i.e., ACE1), the X-linked genes (i.e., ACE2) are expressed as homozygous dominant in females and cannot show in males any advantageous heterozygous condition in case of mutations or polymorphic at-risk conditions. Accordingly, in the presence of a lower activity of the ACE2 gene, as for the one associated with the 8790 G-allele, male-carriers are certainly hemizygotes unable to compensate with the 8790 A-allele counterpart. ACE2 G8790A is located at the beginning of the intron 3, which can also affect the gene expression of other genes by alternate splicing, also having a strong linkage disequilibrium with other SNPs (rs1978124 intron 1 and rs714205 intron 16) in the ACE2 gene.

It has been investigated that the ACE1 I/D variant in combination with the ACE2 G8790A transition mutation is prevalent in hypertensive populations. Therefore, it can be seen that patients with

higher ACE1 activity (D/D-genotype) and lower ACE2 activity (GG-females or hemizygous G-males) may make up the susceptible group for hypertension where they already have classical risk factor association, like, old age, dyslipidemia, and diabetes [97]. Perturbed balance between the ACE1/ACE2 along with over activated RAS may generate systemic disorders. About 60% of the ACE1 levels seem to be determined by the ACE1 I/D polymorphism, and interestingly the activity of ACE1 activity is lower in female both normal and in pathological condition. This evidence, together with the observation that the ACE1 I-allele seems to be overrepresented among females, and that the D-allele (associated with a high level of ACE1) seems more prone to express even higher levels among males, suggests a higher chance of having ACE1/ACE2 imbalance among males during ACE2 receptor suppression, as in the presence of SARS-CoV-2 infection. Balance in ACE/ACE2 receptor mediated entry of angiotensin I and II is the crucial for maintain the balance of Rennin-Angiotensin–aldoterone axis (RAS) to maintain ultimately the vasoconstriction/vasodilation mechanism of the body to potentiate the body homeostasis and blood hemodynamics.

Variation in ACE1 levels can also be modulated by ABO-blood group, locus (9q34.2). ABO polymorphisms (promoter region, and in exon 7) influence the response of treatment within the infected population by ACE inhibitors and can naturally protect the people having O blood group [7].

Receptor Occupancy and Viral Pathogenesis

Both SARS-CoV and SARS-CoV-2 uses same receptor for entry inside the host cell. In humans, a variety of tissues express ACE and ACE2 receptors, but the most prevalent one is lung alveolar tissue. It has been reported that SARS-CoV-2 enters human cells using the SARS-CoV receptor ACE2 and a specific transmembrane serine protease 2 (TMPRSS2) for the spike (S) protein priming [7]. The outer envelope of the SARS-CoV has several spike proteins, which are glycosylated at specific amino acids. The SARS-CoV-2 Spike glycoprotein comprises two subunits, a receptor binding subunit (S1) and a membrane fusion subunit (S2). The Spike glycoprotein assembles into stable homotrimers that together possess 66 canonical sequons for N-linked glycosylation (N-X-S/T, where X is any amino acid except P) as well as several potential O-linked glycosylation sites. It is proposed that, novel corona virus gains a glycan coat which can serve as a glycan shield to facilitate the immunogenicity of the virus. The cryo-EM structure of the SARS-CoV-2 S glycoprotein has been reported recently, which revealed that, like the related protein from the 2002 - 2003 SARS pandemic (SARS-CoV-1), the CoV-2 S protein is also extensively glycosylated [9]. Cryo-EM provides evidence for the existence of 14–16 N-glycans on 22 potential sites in the SARS-CoV-2 S protein and at least 3 O linked glycosylation sites [9]. Glycosylation is important for the specific chemical and physical properties of the protein. It determines the steric hindrance, and thus the specific 3D structure of the protein and therefore important sites for gain of function or loss of function mutation. The N-glycans on S protein play important roles in proper protein folding and priming by host proteases [9]. As glycans can shield the amino acid residues and other epitopes from cells and antibody recognition, glycosylation can enable the coronavirus to evade both the innate and adaptive immune responses [9,10]. The glycosylation of these surface antigens helps the pathogen to bypass the recognition of the virus by host cell receptors except the specific receptor at which it can bind. As in the case of novel corona virus, the glycan shield of the viral spike protein can only recognize the ACE2 receptor site. The glycan shields also perturb the activity of the immune system by cloaking the protein surface from detection by antibodies, and can influence the ability of the host to raise an effective adaptive immune response or even be exploited by the virus to enhance infectivity. The pathogenic SARS-CoV-2 enters human target cells via its viral transmembrane spike (S) glycoprotein. The spike protein is a trimeric class I fusion protein and consists of S1 and S2 subunits. The S1 subunit facilitates the attachment of the virus with the ACE2 receptor, while the S2 subunit allows the fusion of the viral and human cellular membranes.

S1 domain of a corona virus is further divided and classified as N-terminal domain and C-terminal domain. At the C-terminal domain of S1 subunit, there is a 211 amino acid sequence assigned for receptor binding (amino acids 437-529). The receptor binding domain mediates contact with ACE2 receptor. This region of SARS-CoV-2 differs from SARS-CoV by only 5 amino acid residue alterations which are crucial for receptor binding. The residues are Y455L, L486F, N493Q, D494S and T501N. However, a specificity of four amino acid residues from amino acid 482-486 is very crucial with the binding of ACE2 and always gives a firm attachment of SARS-CoV-2 with ACE2. During the viral nucleic acid entry, a proteolytic cleavage activates S2 domain of spike protein, which causes fusion of host cell membrane and viral envelop to facilitate RNA entry inside the host cell. The host proteases, along with transmembrane protease serine protease 2 (TMPRSS2), cathepsin L and furin, take part in the cleavage of the S protein to activate the entry of virus particle with in the host. TMPRSS2 is co-expressed with ACE2 receptor in a variety of tissues like bronchial and lung epithelial cell including nasal epithelial cell which prove the olfactory route of viral entry. The SARS-CoV S protein RBD is projected in a lying down state, which is evidently helpful for immune evasion [11].

SARS-CoV-2 uses ACE2 receptor and human proteases to enter the human cells by fusion of the viral envelop with the host cell membrane. Therefore, drugs that inhibit this fusion procedure can be potential defense mechanism against the virus. Also, it can target the viral envelop to break down, which potentiates its function.

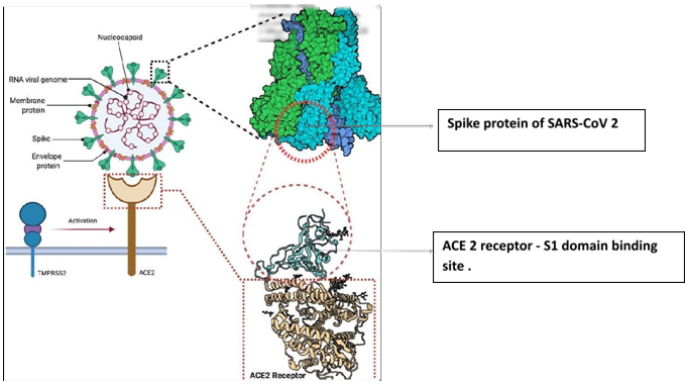

Figure 1: Representative picture of SARS-CoV 2 Spike protein –ACE2 binding (Ref. Wiese et al 2020. Image used with permission)

Ace2 Receptor Polymorphism and Disease Progression

Recent research revealed that ACE2 receptor mutation/polymorphism is directly associated with the severity of COVID-19 diseases. The relationship between the virus binding and subsequent virus entry to the host cell with the peptidase function of ACE2 receptor actually is at the centre of attention. The peptidase activity of ACE2, that regulates renin-angiotensin-aldosterone system in our body, regulating blood pressure and fluid balance. Therefore, hypertension, diabetes and cardiovascular diseases are sought as the primary and most frequent co-morbidity in COVID-19 diseases.

ACE2 limits the adverse vasoconstriction reaction and pro-fibrotic effects of angiotensin II. Hydrolysis of angiotensin II reduces oxidative stress and therefore associated with balanced cardiac function. Disruption of ACE2 results in an increased level of angiotensin II, which may be associated with hypertension, diabetes, dyslipidemia, and ultimately impaired cardiac function and eventual heart failure. Three ACE2 receptor mutations (rs 240157, rs4646155, rs 4830542) have been associated with pathological variation in hypertension. Further, a study from India with hypertensive subjects and age /sex matched normotensive subjects show that one more mutation (C>T) in ACE2 gene (rs 21068809) is associated with clinical outcomes of hypertension. Mutation may affect the rate of expression of ACE 2 gene. In a study with mice model it has been shown that deletion of ACE2 gene is associated with increased circulating angiotensin II, which may potentiate oxidative damage, pathological hypertension and cardiovascular disease. Thus, it can be noted that mutation can affect the function of ACE2, transcription of ACE2. Mutation also affects post transcriptional or post translational medication of ACE2 receptor and perturbation of the function of ACE2 receptor which may be presented as uncontrolled hypertension and cardiovascular diseases. In a Brazilian population, two more mutations in ACE2 gene have been identified (ACE1/D and ACE2 (G8790A). Combination of these two variants increases the susceptibility to pathological hypertension. SNPs in the coding sequences of ACE2 gene have also been identified. One truncated variation has been identified by Cao and colleagues in a Chinese population based study where truncation at Glu300 is projected. In addition, they have reported 32 other variants in ACE2 receptor gene with 7 identified hotspots [12].

During binding with viral S1 domain the ACE 2 receptor protein recruits a 71 amino acid sequence (424-494) of S1 and it is involved in the direct contact with first α helix and Lys 353 with its proximal residues at the N terminus of β sheet 5 of ACE2. A point mutation Leu 584Ala in ACE2 causes significant facilitation of viral entry into the host cell [12].

SARS-CoV-2 Polymorphism

So far, several point mutations and deletions have been identified in entire SARS-CoV-2 genome. Among these, some are closely associated with the disease severity of COVID-19. Several of these mutations are in the receptor binding site and therefore can facilitate or inhibit virus entry to the host cell and these, in turn, alter the virulence. 220 SARS-CoV-2 genomic sequences were identified and studied for mutation analysis, which revealed 8 novel mutational hotspots, among which 5 were predominantly observed in Europe and 3 were in America, whereas none of these were identified in Asia. They have also identified a novel viral gene mutation in RNA dependent RNA polymerase (RdRp) locus which is closely associated with a higher number of point mutations in those mutant strains compared to strains identified from Asia [13]. The most predominant deletion mutation observed is deletion 382, which is observed in several cases in Singapore. This mutation causes truncation of ORF7b and eliminates ORF8 transcription regulatory region from the transcribing viral gene and eventually eliminates ORF8 transcription. Interestingly, this variant was found more pronounced during the early phase of the pandemic, but after March 2020, this was not found efficiently. Instead, a similar del 382 was observed and identified in Wuhan, China in February 2020 along with several other deletion mutations throughout the world (Del 345 in Bangladesh, Del 138 in Australia, Del 62 in Spain and many more) [14]. These deletions are quite obvious and can occur in nature. For example, we can point out the deletion mutations of SARS-CoV seen in 2002- 2003. Scientists frequently observed a deletion of 29 nucleotides during SARS-CoV outbreak, which was associated with severity of acute respiratory syndrome. This deletion was also observed at ORF 8. Larger deletions like 82 nucleotides and 415 nucleotide deletion were reported from SARS-CoV and clarified the association with differential degree of respiratory illness [14]. However, the 29 deletion in ORF8 in SARS-CoV had been associated with slow replication compared to the wild type virus. Therefore, ORF8 must have some role in disease progression in both SARS-CoV-2 and SARS-CoV. Researchers have documented the association of ORF8 in SARS-CoV-2 with immune evasion by down regulating MHC-I molecules. ORF-8 links with the cellular proteins involved in glycoprotein metabolism to work in intracellular molecular cross talk cascade for facilitation

of drug entry and subsequent activation of target molecules [14]. One more prominent mutation has been identified is D614G mutation. This variant with Spike G614 has replaced D 614 as the dominant pandemic form. Consistent increase of G614 in the population increases the infectivity and severity of the disease as it is noted that G614 is associated with lower Ct value, suggestive of higher infectivity and viral load. G614 variants grow rapidly to attain a high titre as the pseudo-typed virion [15]. This D614G mutation causes more efficient binding with ACE2 receptor, less S1 domain shedding and higher s protein incorporation on the virion envelope [16]. This mutation has been found about 29% of the affected population worldwide and in still higher ratio in Europe. One interesting fact is that D614G mutation is frequently associated with a second mutation on the spike protein gene, and it is C- T mutation at the 3,037th position and a third one at the position 241 (C-T) in the 5' untranslated region. One more C-T transition has been identified at bp14,409, at RNA dependent RNA polymerase gene, although not associated with D614G mutation [16]. A very recent review by Guru Prasad pointed out about 400 distinct mutational hotspots on the spike protein sequence [17]. The RBD of spike protein also has over 40 mutational hotspots, which can modulate the binding of S1 with ACE2 receptor. Computational bioinformatics study analyzed about 18354 mutations in S protein and it revealed that most of those mutations can destabilize the entire S protein and RBD. Specially, residue G431, S514 and D614G are important to the overall thermodynamic stability of S protein and RBD. Some of the NH2 terminal linked glycosylation site mutation of S protein can stabilize it further and may increase the infectivity of the virion. In silico analysis of mutation revealed 3705 mutations in SARS-CoV-2 RBD and 11324 mutations in human ACE2. It is also found that SARS-CoV-2 neighbor residues G496 and F497 and D355 and Y41 of ACE2 are crucial for viral S protein and ACE2 interaction [10].

Two conceptual mechanisms can explain the increased infectivity of D614G. According to the first one, D614 is located on the surface of spike protein promoter and interacts easily with the neighboring promoter. Cryo EM structure showed that this side chain of D614 form hydrogen bond with T859 of the neighboring promoter [9]. This promoter- promoter interaction actually occurs near one promoter of S1 unit to the promoter of S2 unit which further facilitate the binding of S1 and S2. Whereas, the 614 G form diminishes the binding interaction of S1 and S2 facilitating the shedding of S1 from S2 subunit and thus promotes S2 entry within the host cell. Alternatively, it could be possible that D614G mutation may alter the binding affinity of RBD with ACE2. The 'up' position of RBD is essential for ACE2 binding and mutation may facilitate the up and down position of the RBD allosterically. In D614, the RBD lies in its 'down' position, which can down regulate the S2 entry within the host cell compared to its 'up' position seen in 614G form [15].

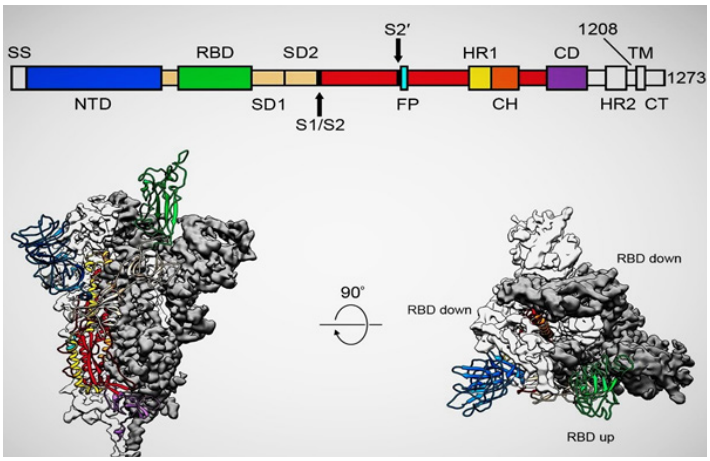

Figure 2: Genetic Configuration of different domain of SARS-CoV 2 and the 3D structure of RBD in its Up and Down form. (Ref: Wrapp et al 2020. Image used with permission.)

Disease severity linked with mutation: Mutations in spike protein of the virus may alter the immunogenicity. The disease severity may be increased or decreased depending on the altered immunogenicity of the virus, as well as increased or decreased viral load within the body. Global tracking data showed that G614 variant spread more rapidly than D614, as it was stated that G614 variants also have a lower Ct value. This confers that G614 variant can also higher viral load. Recent studies reveal that, although G614 is associated with increased viral load and decreased Ct values, it is not associated with greater severity. The status of hospital stay (number of days in hospital, ICU admission, ventilation occupancy) is not significantly associated with G614 variant, although a higher fatality rate has been reported across the countries in case of G614 variants. This higher fatality rate can be further judged with a differential treatment facility, availability of testing and care facilities across the countries. Now, above all, the question is, does this mutation/mutations provoke altered immunological consequences? G614 variant is sensitive to neutralizing antibody, mostly polyclonal antibodies, produced in the body as a response to infection. Till to date, it is not clear that whether D614 and G614 forms of SARS-CoV-2 are differentially activated by neutralizing serum or vaccine produced against the D614 form. If the vaccine produced is not efficiently neutralize the G614 form, then it may require a higher level of vaccine administration to produce a higher level of neutralizing antibody against the G614 form.

The hypothesis that is based on the structure of spike protein can explain the increased infectivity of D614G mutation. D614 is on the surface of the spike protein protomer, 9 of the neighboring, which enables it to contact easily with other protomer living nearby. The side chain of the D614G can form a hydrogen bond with T859 of the neighboring protomer. This protomer-protomer interaction may strengthen the binding of S1 protein of one unit and S2 protein of the other unit. These two sites in the spike protein

bracketing both the dibasic furin and S2 cleavage unit promotes shedding of S1 from viral body. Alternatively, D614G mutation may affect RBD- ACE2 interaction. RBD has to be positioned in a 'Up' to interact with ACE2 receptor. Normally, RBD shows a 'Down', which is not as facilitator as the 'Up' configuration of RBD. When mutation promotes the up configuration in the spike structure, its infectivity increases because of an increase in binding capacity with the ACE2.

Further, Young and coworkers had studied on 282 patients the severity of SARS-CoV-2 and mutation at the ORF8. A 382 bp deletion at ORF is associated with less frequent need of supplemental oxygen and ICU set up than the wild type virus infection. Therefore, researchers associate Δ 382 with milder symptoms compared with the wild type variation of the virus Young et al. 2020) [14].

Neandertals lived in Africa several hundred years' ego gained some environmental adaptation to well acclimatize in that region. Although Neandertals and their Asian sister group Denisovans become extinct about 40,000 years ago but transfer of gene pull through ancestors may contribute to modern human population. Some of these contributions may reflect extreme adaptations to infectious diseases, which are strong selective factors for human generations. Indeed, several genetic variants contributed by arctic hominins to modern humans, which affect genes involved in immunity. As an example, we can point out the existence of a variant of toll-like receptor gene which decreases the susceptibility of Helicobacter pylori infection and the risk of allergies. Second, we can display the role of proteins, interacting with RNA viruses, encoded by DNA regions introgressed from Neandertals profusely. recent studies associate that a haplotype in a region on chromosome 3 with severe illness when infected with SARS-CoV-2. Presence of this haplotype increases the frequency of requiring intensive care in SARS-CoV-2 positive patients almost double. It reaches carrier frequency in South Asia about 65%, 16% in Europe whereas almost nil in East Asia. Therefore, although the haplotype is detrimental for SARS-CoV-2 infected patients, its relative occurrence of frequency actually determines the fate of a particular area over the globe to be severely infected with SARS-CoV-2. The seven loci present on chromosome 6, 12, 19 and 21 harbor Neanderthal like alleles. Presentation of such alleles varies between different geographical locations around the globe and confers the relative severity of disease from SARS-CoV-2 infection. Each copy of the Neandertal haplotype reduces the relative risk of admitting infected patients to intensive care by almost 22%. This Neanderthal haplotype is completely absent in African populations south of the Sahara but exists at an about 25-30% frequency in Eurasia, whereas, in America, it exists in absolute low frequency which can justify the severity among people of America. The protective haplotype presents on the chromosome number 12 has been identified and the regions of such protections are within OAS1, OAS2 and OAS3 gene which are encoding oligoadenylate synthetases. These enzymes are actually activated by ds RNA while induced by viral interferons. They produce short chain polyadenylates which induce ribonuclease L. This ribonuclease potentiates the degradation of intracellular RNA and also initiates various intracellular antiviral immunological mechanisms to check the infection [1-61].

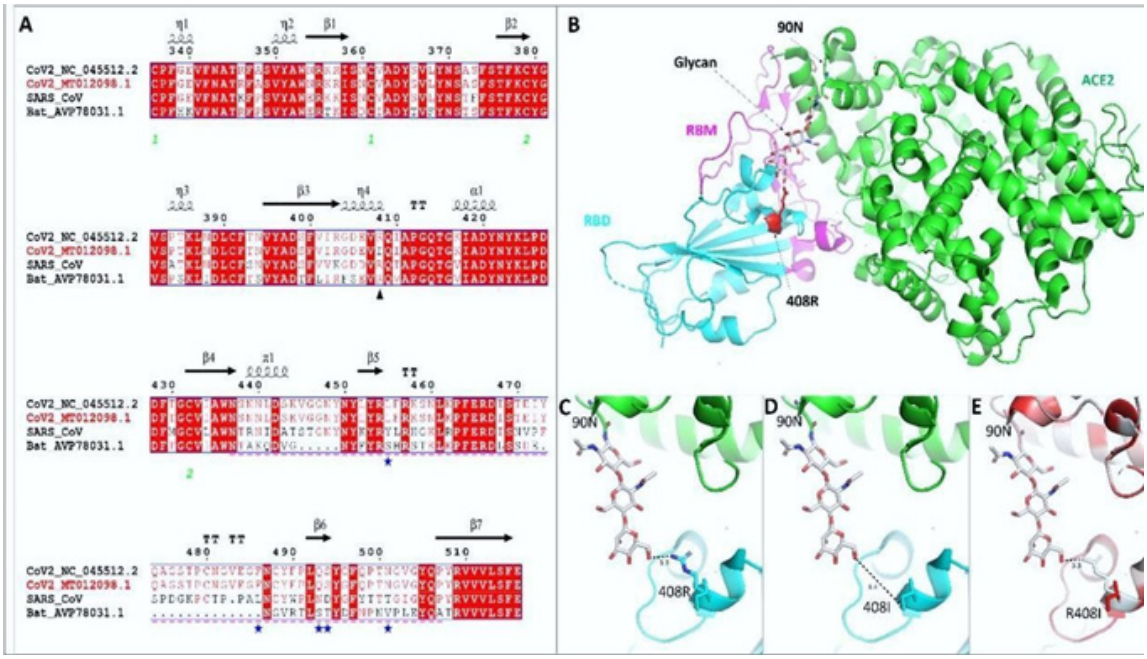

Figure 3: Mutational HotSpot on SARS-CoV2 and possible effects on Spike protein –ACE2 interaction. (Ref: Yong et al 2020. Image used with permission)

Acknowledgement

Constant support and technical help from Dept of Physiology SNGGDCG, Mrs Reshma Mukherjee, and Ms Orchishma Mukherjee is highly solicited.

Conflict of interest

No conflicts of interest exist.

References

1. Annoni, A., Gregori, S., Naldini, L., & Cantore, A. (2019). Modulation of immune responses in lentiviral vector- mediated gene transfer. Cellular Immunology, 342, 103802.
2. Bao, L., Deng, W., Huang, B., Gao, H., Liu, J., Ren, L., Wei, Q., Yu, P., Xu, Y., Qi, F., Qu, Y., Li, F., Lv, Q., Wang, W., Xue, J., Gong, S., Liu, M., Wang, G., Wang, S., … Qin, C. (2020). The pathogenicity of SARS-CoV-2 in hACE2 transgenic mice. Nature, 583(7818), 830–833. https://doi.org/10.1038/s41586-020-2312-y
3. Li, M., Chen, L., Zhang, J., Xiong, C., & Li, X. (2020). The SARS-CoV-2 receptor ACE2 expression of maternal-fetal interface and fetal organs by single-cell transcriptome study. PloS one, 15(4), e0230295.
4. Zou, L., Ruan, F., Huang, M., Liang, L., Huang, H., Hong, Z., ... & Wu, J. (2020). SARS-CoV-2 viral load in upper respiratory specimens of infected patients. New England journal of medicine, 382(12), 1177-1179.
5. Oudit, G. Y., Kassiri, Z., Jiang, C., Liu, P. P., Poutanen, S. M., Penninger, J. M., & Butany, J. (2009). SARS-coronavirus modulation of myocardial ACE2 expression and inflammation in patients with SARS. European journal of clinical investigation, 39(7), 618-625.
6. Burrell, L. M., Burchill, L., Dean, R. G., Griggs, K., Patel, S. K., & Velkoska, E. (2012). Chronic kidney disease: cardiac and renal angiotensin-converting enzyme (ACE) 2 expression in rats after subtotal nephrectomy and the effect of ACE inhibition. Experimental physiology, 97(4), 477-485.
7. Gemmati, D., Bramanti, B., Serino, M. L., Secchiero, P., Zauli, G., & Tisato, V. (2020). COVID-19 and individual genetic susceptibility/receptivity: role of ACE1/ACE2 genes, immunity, inflammation and coagulation. Might the double X-chromosome in females be protective against SARS-CoV-2 compared to the single X-chromosome in males?. International journal of molecular sciences, 21(10), 3474.
8. Gómez, J., Albaiceta, G. M., García-Clemente, M., López-Larrea, C., Amado-Rodríguez, L., Lopez-Alonso, I., ... & Coto, E. (2020). Angiotensin-converting enzymes (ACE, ACE2) gene variants and COVID-19 outcome. Gene, 762, 145102.
9. Wrapp, D., Wang, N., Corbett, K. S., Goldsmith, J. A., Hsieh, C.-L., Abiona, O., Graham, B. S., & McLellan, J. S. (2020). Cryo-EM structure of the 2019-nCoV spike in the prefusion conformation. Science, 367(6483), 1260–1263. https://doi.org/10.1126/science.abb2507
10. Teng, S., Sobitan, A., Rhoades, R., Liu, D., & Tang, Q. (2021). Systemic effects of missense mutations on SARS-CoV-2 spike glycoprotein stability and receptor-binding affinity. Briefings in bioinformatics, 22(2), 1239-1253.
11. Hu, B., Guo, H., Zhou, P., & Shi, Z.-L. (2020). Characteristics of sars-cov-2 and COVID-19. Nature Reviews Microbiology, 19(3), 141–154. https://doi.org/10.1038/s41579-020-00459-7
12. Devaux, C. A., Rolain, J. M., & Raoult, D. (2020). ACE2 receptor polymorphism: Susceptibility to SARS-CoV-2, hypertension, multi-organ failure, and COVID-19 disease outcome. Journal of Microbiology, Immunology and Infection, 53(3), 425-435.
13. Biswas, S. K., & Mudi, S. R. (2020). Genetic variation in SARS-CoV-2 may explain variable severity of COVID-19. Medical hypotheses, 143, 109877.
14. Young, B. E., Fong, S.-W., Chan, Y.-H., Mak, T.-M., Ang, L. W., Anderson, D. E., Lee, C. Y.-P., Amrun, S. N., Lee, B., Goh, Y. S., Su, Y. C. F., Wei, W. E., Kalimuddin, S., Chai, L. Y. A., Pada, S., Tan, S. Y., Sun, L., Parthasarathy, P., Chen, Y. Y. C., … Ng, L. F. P. (2020). Effects of a major deletion in the SARS-CoV-2 genome on the severity of infection and the inflammatory response: An observational cohort study. The Lancet, 396(10251), 603–611. https://doi.org/10.1016/s0140-6736(20)31757-8
15. Korber, B., Fischer, W. M., Gnanakaran, S., Yoon, H., Theiler, J., Abfalterer, W., ... & Montefiori, D. C. (2020). Tracking changes in SARS-CoV-2 spike: evidence that D614G increases infectivity of the COVID-19 virus. Cell, 182(4), 812-827.
16. Zhang, Lizhou, Cody B. Jackson, Huihui Mou, Amrita Ojha, Haiyong Peng, Brian D. Quinlan, Erumbi S. Rangarajan et al. "SARS-CoV-2 spike-protein D614G mutation increases virion spike density and infectivity." Nature communications 11, no. 1 (2020): 1-9.
17. Guruprasad, L. (2021). Human coronavirus spike protein-host receptor recognition. Progress in biophysics and molecular biology, 161, 39-53.
18. Ali, F., Elserafy, M., Alkordi, M. H., & Amin, M. (2020). ACE2 coding variants in different populations and their potential impact on SARS-CoV-2 binding affinity. Biochemistry and biophysics reports, 24, 100798.
19. Asselta, R., Paraboschi, E. M., Mantovani, A., & Duga, S. (2020). ACE2 and TMPRSS2 variants and expression as candidates to sex and country differences in COVID-19 severity in Italy. Aging (Albany NY), 12(11), 10087.
20. 1000 Genomes Project Consortium, Auton, A., Brooks, L. D., Durbin, R. M., Garrison, E. P., Kang, H. M., Korbel, J. O., Marchini, J. L., McCarthy, S., McVean, G. A., & Abecasis, G. R. (2015). A global reference for human genetic variation. Nature, 526(7571), 68–74. https://doi.org/10.1038/nature15393
21. Badawi, S., & Ali, B. R. (2021). ACE2 Nascence, trafficking, and SARS-CoV-2 pathogenesis: the saga continues. Human genomics, 15(1), 1-14.
22. Baig, A. M., Khaleeq, A., Ali, U., & Syeda, H. (2020). Evi-

dence of the COVID-19 virus targeting the CNS: tissue distribution, host–virus interaction, and proposed neurotropic mechanisms. ACS chemical neuroscience, 11(7), 995-998.
23. Benetti, E., Tita, R., Spiga, O., Ciolfi, A., Birolo, G., Bruselles, A., ... & Pinto, A. M. (2020). ACE2 gene variants may underlie interindividual variability and susceptibility to COVID-19 in the Italian population. European Journal of Human Genetics, 28(11), 1602-1614.
24. Beyrouti, R., Adams, M. E., Benjamin, L., Cohen, H., Farmer, S. F., Goh, Y. Y., ... & Werring, D. J. (2020). Characteristics of ischaemic stroke associated with COVID-19. Journal of Neurology, Neurosurgery & Psychiatry, 91(8), 889-891.
25. Burrell, L. M., Harrap, S. B., Velkoska, E., & Patel, S. K. (2013). The ACE2 gene: its potential as a functional candidate for cardiovascular disease. Clinical science, 124(2), 65-76.
26. Chaudhary, M. (2020). COVID-19 susceptibility: potential of ACE2 polymorphisms. Egyptian Journal of Medical Human Genetics, 21(1), 1-8.
27. Gong, Y.-N., Tsao, K.-C., Hsiao, M.-J., Huang, C.-G., Huang, P.-N., Huang, P.-W., Lee, K.-M., Liu, Y.-C., Yang, S.-L., Kuo, R.-L., Chen, K.-F., Liu, Y.-C., Huang, S.-Y., Huang, H.-I., Liu, M.-T., Yang, J.-R., Chiu, C.-H., Yang, C.-T., Chen, G.-W., & Shih, S.-R. (2020). SARS-CoV-2 genomic surveillance in Taiwan revealed novel ORF8-deletion mutant and clade possibly associated with infections in Middle East. Emerging Microbes & Infections, 9(1), 1457–1466. https://doi.org/10.1080/22221751.2020.1782271
28. Grant, O. C., Xue, X., Ra, D., Khatamian, A., Foley, B. L., & Woods, R. J. (2016). Gly-Spec: a webtool for predicting glycan specificity by integrating glycan array screening data and 3D structure. Glycobiology, 26(10), 1027-1028.
29. Imai, Y., Kuba, K., Rao, S., Huan, Y., Guo, F., Guan, B., ... & Penninger, J. M. (2005). Angiotensin-converting enzyme 2 protects from severe acute lung failure. Nature, 436(7047), 112-116.
30. Imai, Y., Kuba, K., & Penninger, J. M. (2008). The discovery of angiotensin-converting enzyme 2 and its role in acute lung injury in mice. Experimental physiology, 93(5), 543-548.
31. Kuba, K., Imai, Y., Ohto-Nakanishi, T., & Penninger, J. M. (2010). Trilogy of ACE2: A peptidase in the renin–angiotensin system, a SARS receptor, and a partner for amino acid transporters. Pharmacology & therapeutics, 128(1), 119-128.
32. Li, Q., Guan, X., Wu, P., Wang, X., Zhou, L., Tong, Y., Ren, R., Leung, K. S. M., Lau, E. H. Y., Wong, J. Y., Xing, X., Xiang, N., Wu, Y., Li, C., Chen, Q., Li, D., Liu, T., Zhao, J., Liu, M., … Feng, Z. (2020). Early transmission dynamics in Wuhan, China, of novel coronavirus–infected pneumonia. New England Journal of Medicine, 382(13), 1199–1207. https://doi.org/10.1056/nejmoa2001316
33. Li, W., Moore, M. J., Vasilieva, N., Sui, J., Wong, S. K., Berne, M. A., ... & Farzan, M. (2003). Angiotensin-converting enzyme 2 is a functional receptor for the SARS coronavirus. Nature, 426(6965), 450-454.
34. Lu, R., Zhao, X., Li, J., Niu, P., Yang, B., Wu, H., Wang, W., Song, H., Huang, B., Zhu, N., Bi, Y., Ma, X., Zhan, F., Wang, L., Hu, T., Zhou, H., Hu, Z., Zhou, W., Zhao, L., … Tan, W. (2020). Genomic characterisation and epidemiology of 2019 novel coronavirus: Implications for virus origins and receptor binding. The Lancet, 395(10224), 565–574. https://doi.org/10.1016/s0140-6736(20)30251-8
35. Lukassen, S., Chua, R. L., Trefzer, T., Kahn, N. C., Schneider, M. A., Muley, T., ... & Meister, M. (2020). Veith C, Boots AW, Hennig BP, Kreuter M, Conrad C, Eils R. SARS-CoV-2 receptor 12 ACE2 and TMPRSS2 are primarily expressed in bronchial transient secretory cells. EMBO 13 J, 39, e105114.
36. Mafessoni, F., Grote, S., de Filippo, C., Slon, V., Kolobova, K. A., Viola, B., ... & Pääbo, S. (2020). A high-coverage Neandertal genome from Chagyrskaya Cave. Proceedings of the National Academy of Sciences, 117(26), 15132-15136.
37. Mao, L., Wang, M., Chen, S., He, Q., Chang, J., Hong, C., ... & Hu, B. (2020). Neurological manifestations of hospitalized patients with COVID-19 in Wuhan, China: a retrospective case series study. MedRxiv.
38. Mohammad, A., Marafie, S. K., Alshawaf, E., Abu-Farha, M., Abubaker, J., & Al-Mulla, F. (2020). Structural analysis of ACE2 variant N720D demonstrates a higher binding affinity to TMPRSS2. Life Sciences, 259, 118219.https://doi.org/10.1016/j.lfs.2020.118219
39. Novelli, A., Biancolella, M., Borgiani, P., Cocciadiferro, D., Colona, V. L., D'Apice, M. R., Rogliani, P., Zaffina, S., Leonardis, F., Campana, A., Raponi, M., Andreoni, M., Grelli, S., & Novelli, G. (2020). Analysis of ACE2 genetic variants in 131 Italian sars-cov-2 positive patients. Research Square Platform LLC. http://dx.doi.org/10.21203/rs.3.rs-39011/v3
40. Pinheiro, D. S., Santos, R. S., Jardim, P. C. V., Silva, E. G., Reis, A. A., Pedrino, G. R., & Ulhoa, C. J. (2019). The combination of ACE I/D and ACE2 G8790A polymorphisms revels susceptibility to hypertension: A genetic association study in Brazilian patients. PloS one, 14(8), e0221248.
41. Plante, J. A., Liu, Y., Liu, J., Xia, H., Johnson, B. A., Lokugamage, K. G., ... & Shi, P. Y. (2021). Spike mutation D614G alters SARS-CoV-2 fitness. Nature, 592(7852), 116-121.
42. Ramchand, J., Patel, S. K., Srivastava, P. M., Farouque, O., & Burrell, L. M. (2018). Elevated plasma angiotensin converting enzyme 2 activity is an independent predictor of major adverse cardiac events in patients with obstructive coronary artery disease. PloS one, 13(6), e0198144.
43. Ramchand, J., Patel, S. K., Srivastava, P. M., Farouque, O., & Burrell, L. M. (2018). Elevated plasma angiotensin converting enzyme 2 activity is an independent predictor of major adverse cardiac events in patients with obstructive coronary artery disease. PloS one, 13(6), e0198144.
44. Shang, J., Ye, G., Shi, K., Wan, Y., Luo, C., Aihara, H., Geng, Q., Auerbach, A., & Li, F. (2020). Structural basis of receptor recognition by SARS-CoV-2. Nature, 581(7807), 221–224. https://doi.org/10.1038/s41586-020-2179-y

45. Shikov, A. E., Barbitoff, Y. A., Glotov, A. S., Danilova, M. M., Tonyan, Z. N., Nasykhova, Y. A., ... & Glotov, O. S. (2020). Analysis of the spectrum of ACE2 variation suggests a possible influence of rare and common variants on susceptibility to COVID-19 and severity of outcome. Frontiers in genetics, 1129.
46. Sinha Dutta S. Genetic variants of human ACE2 are differentially recognized by SARS-CoV-2. BioRxiv.2020.
47. Srivastava, A., Bandopadhyay, A., Das, D., Pandey, R. K., Singh, V., Khanam, N., ... & Chaubey, G. (2020). Genetic association of ACE2 rs2285666 polymorphism with COVID-19 spatial distribution in India. Frontiers in genetics, 1163.
48. Thompson, R. (2020). Pandemic potential of 2019-nCoV. The Lancet Infectious Diseases, 20(3), 280.
49. Walls, A. C., Xiong, X., Park, Y.-J., Tortorici, M. A., Snijder, J., Quispe, J., Cameroni, E., Gopal, R., Dai, M., Lanzavecchia, A., Zambon, M., Rey, F. A., Corti, D., & Veesler, D. (2020). Unexpected receptor functional mimicry elucidates activation of coronavirus fusion. Cell, 183(6), 1732. https://doi.org/10.1016/j.cell.2020.11.031
50. Wang, K., Gheblawi, M., & Oudit, G. Y. (2020b). Angiotensin converting enzyme 2. Circulation, 142(5), 426–428. https://doi.org/10.1161/circulationaha.120.047049
51. Watanabe, Y., Allen, J. D., Wrapp, D., McLellan, J. S., & Crispin, M. (2020). Site-specific analysis of the SARS-CoV-2 glycan shield. BioRxiv.
52. Watanabe, Y., Bowden, T. A., Wilson, I. A., & Crispin, M. (2019). Exploitation of glycosylation in enveloped virus pathobiology. Biochimica et Biophysica Acta (BBA)-General Subjects, 1863(10), 1480-1497.
53. World Health Organization. Coronavirus disease 2019 (COVID-19). Situation report-51. 2020.
54. World Health Organization. Coronavirus disease 2019 (COVID-19). Situation report-75. 2020.
55. Wiese, O., Zemlin, A. E., & Pillay, T. S. (2020). Molecules in pathogenesis: Angiotensin converting enzyme 2 (ACE2). Journal of Clinical Pathology, 74(5), 285–290. https://doi.org/10.1136/jclinpath-2020-206954
56. Wu, A., Peng, Y., Huang, B., Ding, X., Wang, X., Niu, P., ... & Jiang, T. (2020). Genome composition and divergence of the novel coronavirus (2019-nCoV) originating in China. Cell host & microbe, 27(3), 325-328.
57. Yong J, Shen G, Zhang Y et al. Analysis of the mutation dynamics of SARS-CoV-2 reveals the spread history and emergence of RBD mutant with lower ACE2 binding affinity. bioRxiv. 2020. doi: https://doi.org/10.1101/2020.04.09.034942
58. Yuan, Y., Cao, D., Zhang, Y., Ma, J., Qi, J., Wang, Q., ... & Gao, G. F. (2017). Cryo-EM structures of MERS-CoV and SARS-CoV spike glycoproteins reveal the dynamic receptor binding domains. Nature communications, 8(1), 1-9.
59. Hugo, Z., & Svante, P. (2020). The major genetic risk factor fo r severe COVID-19 is inherited from Neandertals. Nature, 587(7835), 610-612.
60. Zeberg, H., & Pääbo, S. (2021). A genomic region associated with protection against severe COVID-19 is inherited from Neandertals. Proceedings of the National Academy of Sciences, 118(9).
61. Zhao, P., Praissman, J. L., Grant, O. C., Cai, Y., Xiao, T., Rosenbalm, K. E., ... & Wells, L. (2020). Virus-receptor interactions of glycosylated SARS-CoV-2 spike and human ACE2 receptor. Cell host & microbe, 28(4), 586-601.

Copyright: *©2022 Sarmishtha Chanda. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.*