

## New COVID-19 Strain (SARS-Cov-2 VUI 202012/01): Lurking Challenge

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**Abstract:** As from the first outbreak of COVID-19 the clinical feature of COVID-19 had been changing progressively. Various data had been reported viral intra and inter-host evolution favoring the mild COVID-19 strains are being accumulating, for better understanding the evolution of COVID-19. So it is essential to investigate the genetic and phenotypic features of this virus. COVID-19 consist various variant so larger clades had been produced and various distinct nomenclature had been proposed. In December 2020 Global Initiative on Sharing All Influenza Data (GISAID) named Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) as hCoV-19 and next strain identified five clades and after that investigators identified 5 lineages (A, B, B.1, B.1.1, B.1.777). The new variant of COVID-19 identified SARS-Cov-2 VUI 202012/01 had been known of lineage B.1.777. COVID-19 associated new variant SARS-CoV-2 VUI (Variant Under Investigation) 202012/01 contains a series of mutation had been described in United Kingdom (UK) and had been found to be highly prevalent in Southeast England and London. All of this mutation is found to be more frequently transmissible than various other strains of COVID-19 Scientist are currently working on vaccine which will protect people against this virus. Currently there is not any evidence about this variant causing more serious illness or higher risk of mortality.

**Keywords:** COVID-19, Strain, Lineage, Mutation, Transmissible.

### INTRODUCTION

It had been emerged as a major pandemic various studies are going on for understanding this disease through genome analysis. Genome of this virus consist structural protein spike, envelope, membrane and nucleocapsid among all of them spike protein is majorly involve in understanding the COVID-19 associated disease mechanism. Spike protein contains furin cleavage site which is not found in other strain of coronaviruses, furin is an enzyme encoded through gene furin. Few proteins are firstly inactive when synthesized furin helps in activating these types of protein by cleaving them and activating the protein [1-3]. It was named furin because it was present in upstream region of oncogene known as FUR (FES upstream region) also known as PACE (Paired basic amino acid cleavage enzyme). Furin enzyme in COVID-19 enhances the receptor affinity and facilitate the membrane fusion.

### Structure analysis of COVID-19

As COVID-19 had been associated with B lineage, it is non-segmented enveloped positive and single stranded RNA virus with 5' cap and 3'poly A tail for performing translation of replicase polyprotein [4]. As COVID-19 genome analysis had revealed that it consist of four structural protein spike (S), envelope

(E), membrane (M), nucleocapsid (N) and various non-structural protein [5]. Among all of the known RNA virus COVID-19 genome is largest [6]. The genome organization of COVID-19 contains ORF1ab- S-ORF 3a-E-ORF6a-ORF7a-ORF8-N-ORF10 from 5'to 3' and do not contains hemagglutinin-esterase (HE) gene which had been found in other strains of coronavirus [7]. About 2/3 of COVID-19 genome consist of ORF1a/b with 16 non-structural proteins considered as largest open reading frame (ORF) remaining 1/3 genome consist structural and accessory proteins [8]. As other viruses genetic diversity is the important source for SARS-CoV-2.

### Genomic mutations

As it was seen by many investigators that mutation and recombination are the main source of genetic diversity for this virus [9]. In this context 9 recombinant pattern had been identified for COVID-19 genome with 6 crucial recombinant regions in spike protein and 1 in RNA dependent RNA polymerase (RdRp), non-structural protein (nsp13) and ORF3a [10]. According to GISAID the virus of COVID-19 mutate at the rate of  $8 \times 10^{-4}$  nucleotides per year [11]. The mutation distribution of COVID-19 pattern had shown the difference in age, geography and time [12]. Various

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genes of COVID-19 are found to be mutated as revealed by various studies in inclusion of ORF 1 ab,3a,6,7,8,10 and spike, membrane, envelope and nucleoprotein [13]. In between them nsp1,2,3,15 of ORF1ab, spike and ORF8 genes were found to be mutated more than other genes [14, 15]. In ORF1ab two insertions with not known effects were found. On the basis of hydrophobicity and charge of amino acid residue in knowing the function of protein for detecting homoplasmic sites of COVID-19, on ORF1ab and N genes were remarkably more than hydrophilic mutation and also on spike protein neutral hydrophobic changes were seen more [16]. The mutational profiles associated analysis had revealed strong mutation towards uracil rich region of genome it may happen due to higher expression and stability of mRNA [17].

COVID-19 associated genetic observation showed that it had been found homologous with other strains of coronavirus SARS-CoV-2 and Middle East Respiratory Syndrome (MERS-CoV-2), 79%, 50% respectively. COVID-19 associated nucleotide sequence had revealed mutation in total 1,516 across the whole genome of virus in comparison other strain of virus. The region of spike protein consist 382 nucleotide mutations in spike protein. Total 1247 nucleotide were found to be mutated in open reading frame among them missense mutation were found in 503 nucleotides. Beside all this substitution of 11 amino acid were seen in spike protein at receptor binding domain. Distinct mutation in spike protein of COVID-19 had been identified in India. Also amino acid 12 and 5 were identified in heptad repeat of amino acid 1 and 2, S2 subunit of spike protein and in topological domain 1-18 and 71-78 of membrane protein 5 amino acid are found to be replaced. Other protein envelope and nucleoprotein had shown replacement of 10 and 75 amino acid respectively.

Various studies revealed the molecular knowledge of neutralizing cross-reacting antibody CR3022 with ambiguous epitope 23 (epitope which is hidden and processed for presenting more efficient result of inflammatory immune response) it is conserved domain for COVID-19 receptor associated binding for spike protein. Only one amino acid had been found to be replaced among 28 residue of epitope of COVID-19 strain this type of mutation is only present in COVID-19 strain revealed through various studies. It could probably happen due to sequence error or evolutionary selection of this strain. Spike protein associated binding with Angiotensin-converting enzyme 2 (ACE-2) do not have any mutational variation. The nucleotide variation 1,516 was revealed through genome analysis at different position in entire genome for the COVID-19 strain, nucleotide excision was recognized at 12 sites of entire genome beside the ORF-8 and ORF7 mainly in polyprotein coding sequence. All of these mutation and protein profile analysis had revealed the large number of amino acid replacement which

leads to the diverged proteins of COVID-19 associated viral protein [18].

As there is not any phenotypic data for the variant of COVID-19 but structural analysis of COVID-19 had been identified it consist envelope, membrane, nucleocapsid and spike protein beside all of these protein there are also some accessory protein ORF 3, 6a, 7a, 7b, 8a, 8b, 9b they all are involved in antagonist function of interferon which plays important function in innate immunity so it leads to suppress the immune response [19]. As found in VUI-202012/01 variant spike protein had an important role in COVID-19 infection, spike protein binds to the receptor of host cell and takes entry into it. COVID-19 involved binding to ACE-2 spike protein contains two subunit S1 and S2 function in binding to receptor and S2 performs membrane fusion of host cell and virus. This mechanism is assisted by transmembrane protease serine 2 (TMPRSS2) perform spike protein mediated cleavage facilitate activation of virus and provide one of the important host factor for COVID-19 mediated pathogenicity.

### Virulence

COVID-19 has similarity in structural context to the other strains of coronavirus mainly SARS-COV-1 it had homology with genome and their protein, also host cell associated entry with receptor ACE-2. Majority of studies reported that COVID-19 specific virulence factors are found to be directed in opposition of host immune response. The cytokine storm which is characteristic of innate immune response is usually found in the COVID-19 patients [20]. Various other studies associated data had suggested that virus associated tricking leads to dichotomous immune response hyperactive immune response of leading to cytokine storm consequently leads to decrease in T-cell mediated antiviral functions. Investigators revealed the development of non-adaptive immune response profile associated with COVID-19 severe outcome. Profiling of immune system showed overall elevation in innate cell lineages with association of T-cells. The COVID-19 infected patients were correlated with interferon and cytokines plasma levels [21]. Investigators also noted the association between early, elevated cytokines and poor outcome of disease. The patients with COVID-19 infection also contains moderate disease shown a progressive reduction in antiviral and antifungal innate immune response. Comparatively patients with severe disease shown higher level of response throughout the course of disease. The severity of this disease was also accompanied through elevation in various innate immune response markers in inclusion of interleukins IL-5, IL-13, eosinophils and immunoglobulin E these findings signify the importance of early interventions associated with immunology which targets inflammatory markers which are the prediction of acute symptoms inspite of targeting the cytokine present at the late stage [22].

### SARS-CoV-2 VOC 202012/01

This variant of COVID-19 Variant of Concern 202012/01 (VOC-202012/01) also named as B.1.1.7 was first arise in October 2020 at the time of COVID-19 pandemic in United Kingdom and spread in mid-December. Coronavirus had been changing constantly through mutation and emergence of the variant VUI-202012/01 had occurred and it is not itself a cause of concern. COVID-19 associated diversification due to adaptation and evolution process had been observed worldwide and it is anticipated to occur with currently going on viral transmission in general and particularly for RNA virus [23].

There is not any data available for these variant associated phenotypic features with reference for the antibody associated capacity obtained through under developing stage of vaccination program for neutralizing this variant. As it contains various spike protein sequence associated mutation and receptor binding site. As the vaccine currently developing is based on spike protein sequence so, it is necessary to screen the variation in this protein among circulating COVID-19 strains and its antigenic alteration. Though, it should be crucial to conduct the effectiveness of COVID-19 vaccine, if feasible than variant-determined to virus should be included for estimation. Also one thing had to be remembered that T-cells play important role in protection against COVID-19 and in clearing this infection. So, T-cell mediated immunity should also be assessed both during COVID-19 infection and also during vaccination. Currently there is a lack of evidences for indicating that this virus incidence is restricted to few of the countries or local areas.

### Virulence

A committee named New and Emerging Respiratory Virus Threats Advisory Group (NERVTAG) had conducted a study regarding the severity of this new variant but there is not any sufficient data present till now for getting on any conclusion. On the other side many investigators also showed that this variant contains higher rate mortality this statement was supported by many other investigators also. Many other committees had declared in mid of December 2020 that there is not any evidence present currently which can revealed that this strain can cause more severity or not [24]. Even though it had been diagnosed in vast geography mainly where higher cases are been diagnosed. Also there was not any evidence found for prolonged COVID-19 patients would develop this variant with reason of distinct host response [25]. Many laboratories are running tests for finding evidence for understanding the severity of this new variant. As there are currently many research are going on for this variant so the virulence for this variant is not clear till due to insufficient data and many other reasons.

### Mutation found in VOC 202012/01

The COVID-19 mutates rapidly and acquires one new mutation in its genome within two weeks. The variant of SARS-CoV-2 had been detected recently in United Kingdom in October 2020 and caused due to mutations. As mutations are of many types among them one is silent mutation with not any change in structure of protein they encodes. The protein is translated through genetic codon made up of three codon which translate amino acid which further change to protein. Translation of same amino acid associate with synonymous mutation, other changes in codon leads to change in amino acid is non-synonymous mutation this mutation do not changes the function of protein. The variant of COVID-19 named VOC 202012/01 had found in United Kingdom's recently due to several mutations, among which 14 non-synonymous mutation and 6 were synonymous and 3 deletion. Deletion 69/70 this double deletion had been seen spontaneously various times and it probably lead to change in shape of spike protein. P681H associated mutation is seen at the spike protein subunit S1/S2 at furin cleavage site with higher variability in other coronaviruses, this mutation had been noticed multiple times. Q27 stop mutation is also seen in spike protein but at a distinct gene at ORF8, its function is not known till now. This kind of mutation is seen previously in one strain of coronavirus but disappeared frequently in Singapore.

The focus on mutation is a best and commonly used way of tracking the spread of virus. This variant of COVID-19 is named VUI-202012/10 is been elaborated by 17 mutation among them one most important mutation is seen in asparagine to tyrosine amino acid residue at 501 position mutation is seen in this position because it is inside spike glycoprotein receptor binding domain mainly in receptor binding motif it binds to human and murine angiotensinogen converting enzyme-2 mutant in the RBD could lead to becoming this of virus more infectious [26]. VUI-202012/01 had been identified through genome sequencing of virus in United Kingdom. It had been identified by various mutations in spike protein at (A570D, D614G, D681H, T716I, S982A, and D1118H) also this variant had been seen in people younger than 60 years. Phylogenetic analysis showed few intermediary forms in between VUI-202012/01 variant and other circulating viruses found from GISAID. It differs by 29 nucleotides substitutions from the strain of COVID-19 found in Wuhan. VUI-202012/01 had been known to be substantially more transmissible than other variants but there is not any sufficient data for reaching at any conclusion on mechanism of greater transmissibility. The most important change seen in this variant is at N5014 which results in change of amino acid asparagine to tyrosine at 501 positions. This occurs due to its position inside the receptor binding motif (RBM) which is utilized in binding to human angiotensinogen converting enzyme-2 [27, 28]. Mutation at RNA binding domain (RBD) leads to change in antibody

recognition and binding specificity of ACE-2 [29]. Chan *et al.* reported that N501Y affects the binding affinity of spike protein to receptor and this mutation individually or in combination of deletion at 69/70 in N-terminal domain is increasing the transmissibility of this virus infection [30].

### Structural analysis of VOC 202012/01

As this variant associated phenotypic data is not clearly known yet but as other strains of coronavirus this new variant also contains spike protein associated mutation had been mainly found in this variant. Unusual and higher counts of mutation in spike protein and further genomic analysis of this variant with huge sequencing description in UK suggested that this variant had not arisen by any moderate accumulation of mutation in UK. Also it turns out that this variant could not likely to be emerged from selection pressure from vaccination program which is ongoing it had been observed that this increase do not matched with the activity timing. One description for this known to be extended infection of COVID-19 in an individual patient with reduced immunodefensive power [31, 32]. This leads to accumulation of mutation escaping the immune system response at greater rate. Other investigation had been made that adaption ability of virus in distinct susceptible species of animal and after that transmitting back to human from animal host. All this revealed that this variant with various mutations in spike protein distinct mutation of spike protein have been identified in Netherlands [33]. Also this concludes that probably this variant had been arising from circulation in various places with lower coverage of sequence. This assumption is slightly reasonable; therefore, mutations randomly obtained at the time of circulation of virus and this did not explain the spike protein associated unusual mutations in higher number, undetected circulations were seen for longer time for higher number of mutation to accumulate is also not very certain due to worldwide traveling pattern. Currently the investigation going on in UK for knowing the spread of the VUI-202012/01 across UK and EU/EEA countries. The study in UK is based on evaluation of severity, antigenic change transmissibility with neutralization by serum of patient recovered from the infection. The diagnosis assays includes lateral flow device. Epidemiology and phylodynamic investigations had also being tackle for assessing the elevated transmissibility of this variant with respect of co-circulating viral variants.

### Transmissibility

Most of the mutation identified had not provided the virus with discriminating advantages like elevated transmissibility from higher receptor binding affinity or capability of evading the immune response of host by changing surface structure of antibody. Investigations of D614G variant had identified that a selective advantage had been seen from increased cellular infectivity not any identifiable effect had been

revealed on severity of the virus infection [34]. Among various mutations there is not any evidence that D614G had the characteristic of more quick spread. On the other hand G614 variant spread more frequent in respiratory tract of human epithelial cells conquering D614 virus. There is various evidence about more quick spread of variant G614 in comparison of virus without mutation. The strain VOC202012/01 had been proved to produce more serious illness than other variant of COVID-19. As the vaccine approved by Food and Drug Administration (FDA) are polyclonal (producing antibodies which target spike protein). As the variants found are causing mutation in spike protein for evading immunity induced by vaccine or natural infection. The capability of evading the immunity induced through vaccination may be most important because once vaccination have been performed in a huge proportion then it will result in immune pressure which will lead to emergence of these kind of variant through escape mutant selection. Currently there is not any evidence for this occurrence and most of the investigators trust that these mutants are cannot emerge due to the nature of virus. Additionally RdRp inhibitor also targets other protein of virus which can provide better COVID-19 therapy efficacy for its treatment. Convalescent Plasma Transfusion (CPT) treatment where convalescent sera of recovered people from COVID-19 is injected in COVID-19 patients and increasingly had been used. [35] These mutation and analysis of protein profile shows substitution in amino acids stating SARS-CoV-2 associated with heterogeneous protein[36]As we all are aware of currently known respiratory disease which is caused by severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) known as COVID-19. This disease had progress rapidly and became a global health problem. SARS-CoV-2 associated Nucleotide sequence has approximately 1,516 mutations across the genome of this strain [37].

Comparative analysis of both COVID-19 and its variant VOC 202012/01 revealed that only spike protein had been the main element associated in causing the disease. As the variant is formed due to the mutation in SARS-CoV-2, so hypothesis that COVID-19 is a small bubble consisting various small clubs projecting out from its surface the virus contains distinct types of protein and genetic code called RNA enveloped by a layer of lipid. The small club is known as spike protein. The spike protein attaches to the receptor of host cells and if it binds appropriately the virus can infect the cells. The genetic code which are responsible for making all the protein of coronavirus and changes in genome of virus results in alteration of its amino acid which can leads to the mutaion in spike protein. After the virus infects the host cell it increases its copy number by the process of replication at the time of this process errors occurs which are known to be mutations. As we are aware about SARS-CoV-2 mutates regularly acquiring about one new mutation within every 2

weeks. All of this comparison had been summarized in Table-1.

**Table-1: Comparative analysis of COVID-19 and VOC-202012/01**

Features	COVID-19	VOC-202012/01
<b>Define</b>	It is a contagious disease caused by SARS-CoV-2	It is a variant of COVID-19
<b>Discovered</b>	It was identified in December 2019	Variant was detected in October 2020
<b>Caused</b>	Through respiratory route by the infected person's respiratory droplets or aerosols of during sneezing and coughing	Cause for this variant is similar
<b>Transmission</b>	Various research have been done for understanding the dynamics of transmissibility based on epidemiology models estimating the infected people under given conditions	This variant is found as substantially higher rate of transmission than other variants but till now there is not any sufficient record for this
<b>Virulence</b>	Its virulence explained that the various structural and non-structural features of COVID-19 like furin cleavage site, ORF3b and dynamic conformational changes in structure of spike protein at the time of host cell fusion. This gives it an infectivity and virulence above previous strains of coronaviruses leading to the pandemic. Virulence of COVID-19 had provided the evidence for impact on infectivity and transmissibility of disease severity and development of immune response against the infection in inclusion of vaccines.	Virulence for this variant was concluded by NERVTAG as there is not much sufficient data for reaching to any conclusion in context of severity of this disease. As also there is not any evidence about the severity of this variant leading to death. There is much investigation currently ongoing for knowing the virulence of this variant.
<b>Genome mutation</b>	Genomic analysis of COVID-19 was found to be homologous with previous strains of SARS-CoV. COVID-19 associated mutation was mainly found in their structural protein which was 382 nucleotide and other was found in non-structural proteins and ORF with 1247 nucleotides.	As this variant came into lime light in December 2020 and its genome analysis showed multiple mutation in various genomic region. Mainly this was seen in asparagine to tyrosine amino acid residue at 501 position mutation is seen in this position because it is inside spike glycoprotein receptor binding domain mainly in receptor binding motif it binds to human and murine angiotensinogen converting enzyme-2 mutant in the RBD could lead this virus more infectious

## CONCLUSIONS

The genome of COVID-19 had been studied extensively many efforts had been made for revealing its behavior based on the genetics, variation, mutations. As from various studies it had been every decade outbreak were known SARS in 2000s, 2010s MERS and currently COVID-19. The new variant of COVID-19 VOC 202012/01 came in light at early in December 2020 due to phenomenal genomic structure system of COVID-19. After all the genomic analysis and epidemiology of this variant revealed increased transmission of this variant. All the evidence of investigation currently supports only increased transmission of this variant there is not any evidence for the severity and re-infections impact. As there is not any phenotypic data available for VOC 202012/01 but spike protein is the main protein found to be mutated in both the COVID-19 and VOC 202012/01 and this protein is the basis for vaccination in both the strains. Till now there had been not any data for the patients recovered from COVID-19 had been re-infected with this variant. Although, it may be due to the antibodies develop in the recovered patients of COVID-19 against

this variant. So, we should be reassured that the target of vaccine is entire spike protein and early evidence indicated that immunity after vaccination becomes robust. Additionally, the broad scale antiviral drugs and vaccines based on genetic profile of both virus and host cells are highly approved.

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