Genomic analysis and comparative multiple sequences of SARS-CoV2

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Abstract:-

Background: On December 31, 2019, China announced the outbreak of a new coronavirus in Wuhan, and the virus has since spread globally. The development of antiviral strategies depends on understanding the molecular mechanisms of genome selection we define the correlation of ten coronavirus (SARS-CoV2) sequences from various countries to analyze the genomic patterns of disease origin

Methods: We apply a Clustalw web service for Multiple sequence alignment (https://www.genome.jp/tools-bin/clustalw)and apply genomic analysis to sequence SARS-CoV2 from GenBank(https://www.ncbi.nlm.nih.gov/genbank/)

Results: Using (NCBI) database and genome alignment for protein sequencing found no differences in amino acid sequences between M and N proteins. The spike (S) protein has two amino acid variations.

INTRODUCTION:-

Infections such as (SARS-CoV)and(MERSCoV), which are spread from animals to humans, have caused pneumonia all over the world. SARS was first discovered in Guangdong, China, in 2002, and has since spread around the world, resulting in 8096 infected cases and 774 deaths. Then a coronavirus appeared in Wuhan, named SARS-CoV2 (International Committee on Taxonomy of Viruses). Coronaviruses are classified into four genera (α, β, ρ and δ). SARSCoV2 (2019) and SARS-CoV1 (2003) are belong to coronaviruses. Coronaviruses contain four proteins: The spike (S) protein helps the virus bind to the host cell's membrane, while the N (nucleocapsid) protein protects the virus's RNA genome. The E (envelope) and M (membrane).

the coronavirus start transmission *when attaches to host cell membrane receptors and enters the host cell. The virus genome's RNA gene 1 then starts to replicate. After that, the virus synthesises subgenomic RNAs with new transcription. and make a lot of copy of virus which attack human body and its Lung.(https://youtu.be/5DGwOJXSxqg)*

SARS-CoV1 is thought to spread from bats and civets to humans, where it causes extreme respiratory illness and a 10% mortality rate. Before being transmitted to humans, Wuhan SARS-coV2 is thought to be transmitted from a bat (Ra TG13).

The s protein is consist two subunit s1 and s2 on the s1 there is receptor binding domain is for ability of binding to human cell and effect him it bind with ACE2 which lung is produce (https://youtu.be/OVDaq_vOQ48)

in this paper, We compared SARS-CoV2 sequences from various countries to examine disease origin and evolution genomic providing genomic knowledge for the creation of new control methods against the worldwide SARS-CoV2 pandemic.

Related work:-

- *COVID-19 Genome Analysis using Alignment-Free Methods:-*
  The genome sequences for COVID-19 strains, taken from NCBI's GenBank, are compared and analyzed with AF methods for: (1) extracting frequent patterns of nucleotides in COVID-19 genome sequences, (2) finding the similarity/dissimilarity between COVID-19 genome sequences by using difference distance measure and (3) Phylogeny construction with various AF methods for COVID-19 genome sequence
- *Mutation hot spots in Spike protein of COVID-19:-*
  Multiple sequence alignments were done using alignment tool of NCBI virus server as well as CLUSTAL Omega. Sequence alignments from CLUSTAL Omega was viewed using MView tool. Multiple sequence Alignment of COVID-19 spike protein sequences from United States of America showed multiple mutations at few frequent locations
- *A lot of web service that we can use it to make alignment sequence as :-*
  MAFFT ---------→https://www.genome.jp/tools-bin/mafft
  PRRN----------→https://www.genome.jp/tools-bin/prrn

Methodology:-

. we make genomic analysis to discover covid 19 and focus on evolutionary and phylogenetic analyses have applied in disease. Using Clustalw web service to Multiple Sequence Alignment

E protein alignment

```
UC MYSFVSEETGTLIVNSVLLFLAFVVFLLVTLAILTALRLCAYCCNIVNVSLVKPSFYVYS
A1 MYSFVSEETGTLIVNSVLLFLAFVVFLLVTLAILTALRLCAYCCNIVNVSLVKPSFYVYS
UW MYSFVSEETGTLIVNSVLLFLAFVVFLLVTLAILTALRLCAYCCNIVNVSLVKPSFYVYS
C2 MYSFVSEETGTLIVNSVLLFLAFVVFLLVTLAILTALRLCAYCCNIVNVSLVKPSFYVYS
C1 MYSFVSEETGTLIVNSVLLFLAFVVFLLVTLAILTALRLCAYCCNIVNVSLVKPSFYVYS
T1 MYSFVSEETGTLIVNSVLLFLAFVVFLLVTLAILTALRLCAYCCNIVNVSLVKPSFYVYS
T2 MYSFVSEETGTLIVNSVLLFLAFVVFLLVTLAILTALRLCAYCCNIVNVSLVKPSFYVYS
J2 MYSFVSEETGTLIVNSVLLFLAFVVFLLVTLAILTALRLCAYCCNIVNVSLVKPSFYVYS
J1 MYSFVSEETGTLIVNSVLLFLAFVVFLLVTLAILTALRLCAYCCNIVNVSLVKPSFYVYS
K1 MYSFVSEETGTLIVNSVLLFLAFVVFLLVTLAILTAHRLCAYCCNIVNVSLVKPSFYVYS

UC RVKNLNSSRVPDLLV
A1 RVKNLNSSRVPDLLV
UW RVKNLNSSRVPDLLV
C2 RVKNLNSSRVPDLLV
C1 RVKNLNSSRVPDLLV
T1 RVKNLNSSRVPDLLV
T2 RVKNLNSSRVPDLLV
J2 RVKNLNSSRVPDLLV
J1 RVKNLNSSRVPDLLV
K1 RVKNLNSSRVPDLLV
```

We found one amino acid mutation at "H" from South Korea comparing the "L" from other nine sequences. Yellow line indicates the difference in 10 sequence alignments

M protein alignment

```
J1 MADSNGTITVEELKKLLEQWNLVIGFLFLTWICLLQFAYANRNRFLYIIKLIFLWLLWPV
J2 MADSNGTITVEELKKLLEQWNLVIGFLFLTWICLLQFAYANRNRFLYIIKLIFLWLLWPV
K1 MADSNGTITVEELKKLLEQWNLVIGFLFLTWICLLQFAYANRNRFLYIIKLIFLWLLWPV
T1 MADSNGTITVEELKKLLEQWNLVIGFLFLTWICLLQFAYANRNRFLYIIKLIFLWLLWPV
T2 MADSNGTITVEELKKLLEQWNLVIGFLFLTWICLLQFAYANRNRFLYIIKLIFLWLLWPV
C1 MADSNGTITVEELKKLLEQWNLVIGFLFLTWICLLQFAYANRNRFLYIIKLIFLWLLWPV
C2 MADSNGTITVEELKKLLEQWNLVIGFLFLTWICLLQFAYANRNRFLYIIKLIFLWLLWPV
UW MADSNGTITVEELKKLLEQWNLVIGFLFLTWICLLQFAYANRNRFLYIIKLIFLWLLWPV
A1 MADSNGTITVEELKKLLEQWNLVIGFLFLTWICLLQFAYANRNRFLYIIKLIFLWLLWPV
UC MADSNGTITVEELKKLLEQWNLVIGFLFLTWICLLQFAYANRNRFLYIIKLIFLWLLWPV

J1 TLACFVLAAVYRINWITGGIAIAMACLVGLMWLSYFIASFRLFARTRSMWSFNPETNILL
J2 TLACFVLAAVYRINWITGGIAIAMACLVGLMWLSYFIASFRLFARTRSMWSFNPETNILL
K1 TLACFVLAAVYRINWITGGIAIAMACLVGLMWLSYFIASFRLFARTRSMWSFNPETNILL
T1 TLACFVLAAVYRINWITGGIAIAMACLVGLMWLSYFIASFRLFARTRSMWSFNPETNILL
T2 TLACFVLAAVYRINWITGGIAIAMACLVGLMWLSYFIASFRLFARTRSMWSFNPETNILL
C1 TLACFVLAAVYRINWITGGIAIAMACLVGLMWLSYFIASFRLFARTRSMWSFNPETNILL
C2 TLACFVLAAVYRINWITGGIAIAMACLVGLMWLSYFIASFRLFARTRSMWSFNPETNILL
UW TLACFVLAAVYRINWITGGIAIAMACLVGLMWLSYFIASFRLFARTRSMWSFNPETNILL
A1 TLACFVLAAVYRINWITGGIAIAMACLVGLMWLSYFIASFRLFARTRSMWSFNPETNILL
UC TLACFVLAAVYRINWITGGIAIAMACLVGLMWLSYFIASFRLFARTRSMWSFNPETNILL

J1 NVPLHGTILTRPLLESELVIGAVILRGHLRIAGHHLGRCDIKDLPKEITVATSRTLSYYK
J2 NVPLHGTILTRPLLESELVIGAVILRGHLRIAGHHLGRCDIKDLPKEITVATSRTLSYYK
K1 NVPLHGTILTRPLLESELVIGAVILRGHLRIAGHHLGRCDIKDLPKEITVATSRTLSYYK
T1 NVPLHGTILTRPLLESELVIGAVILRGHLRIAGHHLGRCDIKDLPKEITVATSRTLSYYK
T2 NVPLHGTILTRPLLESELVIGAVILRGHLRIAGHHLGRCDIKDLPKEITVATSRTLSYYK
C1 NVPLHGTILTRPLLESELVIGAVILRGHLRIAGHHLGRCDIKDLPKEITVATSRTLSYYK
C2 NVPLHGTILTRPLLESELVIGAVILRGHLRIAGHHLGRCDIKDLPKEITVATSRTLSYYK
UW NVPLHGTILTRPLLESELVIGAVILRGHLRIAGHHLGRCDIKDLPKEITVATSRTLSYYK
A1 NVPLHGTILTRPLLESELVIGAVILRGHLRIAGHHLGRCDIKDLPKEITVATSRTLSYYK
UC NVPLHGTILTRPLLESELVIGAVILRGHLRIAGHHLGRCDIKDLPKEITVATSRTLSYYK

J1 LGASQRVAGDSGFAAYSRYRIGNYKLNTDHSSSSDNIALLVQ
J2 LGASQRVAGDSGFAAYSRYRIGNYKLNTDHSSSSDNIALLVQ
K1 LGASQRVAGDSGFAAYSRYRIGNYKLNTDHSSSSDNIALLVQ
T1 LGASQRVAGDSGFAAYSRYRIGNYKLNTDHSSSSDNIALLVQ
```

not found any mutation in 10 sequences of M protein region

N protein alignment

```
J1 MSDNGPQNQRNAPRITFGGPSDSTGSNQNGERSGARSKQRRPQGLPNNTASWFTALTQHG
J2 MSDNGPQNQRNAPRITFGGPSDSTGSNQNGERSGARSKQRRPQGLPNNTASWFTALTQHG
K1 MSDNGPQNQRNAPRITFGGPSDSTGSNQNGERSGARSKQRRPQGLPNNTASWFTALTQHG
T1 MSDNGPQNQRNAPRITFGGPSDSTGSNQNGERSGARSKQRRPQGLPNNTASWFTALTQHG
T2 MSDNGPQNQRNAPRITFGGPSDSTGSNQNGERSGARSKQRRPQGLPNNTASWFTALTQHG
C1 MSDNGPQNQRNAPRITFGGPSDSTGSNQNGERSGARSKQRRPQGLPNNTASWFTALTQHG
C2 MSDNGPQNQRNAPRITFGGPSDSTGSNQNGERSGARSKQRRPQGLPNNTASWFTALTQHG
UW MSDNGPQNQRNAPRITFGGPSDSTGSNQNGERSGARSKQRRPQGLPNNTASWFTALTQHG
A1 MSDNGPQNQRNAPRITFGGPSDSTGSNQNGERSGARSKQRRPQGLPNNTASWFTALTQHG
UC MSDNGPQNQRNAPRITFGGPSDSTGSNQNGERSGARSKQRRPQGLPNNTASWFTALTQHG

J1 KEDLKFPRGQGVPINTNSSPDDQIGYYRRATRRIRGGDGKMKDLSPRWYFYYLGTGPEAG
J2 KEDLKFPRGQGVPINTNSSPDDQIGYYRRATRRIRGGDGKMKDLSPRWYFYYLGTGPEAG
K1 KEDLKFPRGQGVPINTNSSPDDQIGYYRRATRRIRGGDGKMKDLSPRWYFYYLGTGPEAG
T1 KEDLKFPRGQGVPINTNSSPDDQIGYYRRATRRIRGGDGKMKDLSPRWYFYYLGTGPEAG
T2 KEDLKFPRGQGVPINTNSSPDDQIGYYRRATRRIRGGDGKMKDLSPRWYFYYLGTGPEAG
C1 KEDLKFPRGQGVPINTNSSPDDQIGYYRRATRRIRGGDGKMKDLSPRWYFYYLGTGPEAG
C2 KEDLKFPRGQGVPINTNSSPDDQIGYYRRATRRIRGGDGKMKDLSPRWYFYYLGTGPEAG
UW KEDLKFPRGQGVPINTNSSPDDQIGYYRRATRRIRGGDGKMKDLSPRWYFYYLGTGPEAG
A1 KEDLKFPRGQGVPINTNSSPDDQIGYYRRATRRIRGGDGKMKDLSPRWYFYYLGTGPEAG
UC KEDLKFPRGQGVPINTNSSPDDQIGYYRRATRRIRGGDGKMKDLSPRWYFYYLGTGPEAG
```

Genomic analysis of N protein amino acid sequence.

We do not observe any mutation in 10 sequences of N protein region

s

S protein alignment

```
J1 MFVFLVLLPLVSSQCVNLTTRTQLPPAYTNSFTRGVYYPDKVFRSSVLHSTQDLFLPFFS
J2 MFVFLVLLPLVSSQCVNLTTRTQLPPAYTNSFTRGVYYPDKVFRSSVLHSTQDLFLPFFS
UW MFVFLVLLPLVSSQCVNLTTRTQLPPAYTNSFTRGVYYPDKVFRSSVLHSTQDLFLPFFS
UC MFVFLVLLPLVSSQCVNLTTRTQLPPAYTNSFTRGVYYPDKVFRSSVLHSTQDLFLPFFS
A1 MFVFLVLLPLVSSQCVNLTTRTQLPPAYTNSFTRGVYYPDKVFRSSVLHSTQDLFLPFFS
T2 MFVFLVLLPLVSSQCVNLTTRTQLPPAYTNSFTRGVYYPDKVFRSSVLHSTQDLFLPFFS
C1 MFVFLVLLPLVSSQCVNLTTRTQLPPAYTNSFTRGVYYPDKVFRSSVLHSTQDLFLPFFS
C2 MFVFLVLLPLVSSQCVNLTTRTQLPPAYTNSFTRGVYYPDKVFRSSVLHSTQDLFLPFFS
T1 MFVFLVLLPLVSSQCVNLTTRTQLPPAYTNSFTRGVYYPDKVFRSSVLHSTQDLFLPFFS
K1 MFVFLVLLPLVSSQCVNLTTRTQLPPAYTNSFTRGVYYPDKVFRSSVLHSTQDLFLPFFS

J1 NVTWFHAIHVSGTNGTKRFDNPVLPFNDGVYFASTEKSNIIRGWIFGTTLDSKTQSLLIV
J2 NVTWFHAIHVSGTNGTKRFDNPVLPFNDGVYFASTEKSNIIRGWIFGTTLDSKTQSLLIV
UW NVTWFHAIHVSGTNGTKRFDNPVLPFNDGVYFASTEKSNIIRGWIFGTTLDSKTQSLLIV
UC NVTWFHAIHVSGTNGTKRFDNPVLPFNDGVYFASTEKSNIIRGWIFGTTLDSKTQSLLIV
A1 NVTWFHAIHVSGTNGTKRFDNPVLPFNDGVYFASTEKSNIIRGWIFGTTLDSKTQSLLIV
T2 NVTWFHAIHVSGTNGTKRFDNPVLPFNDGVYFASTEKSNIIRGWIFGTTLDSKTQSLLIV
C1 NVTWFHAIHVSGTNGTKRFDNPVLPFNDGVYFASTEKSNIIRGWIFGTTLDSKTQSLLIV
C2 NVTWFHAIHVSGTNGTKRFDNPVLPFNDGVYFASTEKSNIIRGWIFGTTLDSKTQSLLIV
T1 NVTWFHAIHVSGTNGTKRFDNPVLPFNDGVYFASTEKSNIIRGWIFGTTLDSKTQSLLIV
K1 NVTWFHAIHVSGTNGTKRFDNPVLPFNDGVYFASTEKSNIIRGWIFGTTLDSKTQSLLIV

J1 NNATNVVIKVCEFQFCNDPFLGVYYHKNNKSWMESEFRVYSSANNCTFEYVSQPFLMDLE
J2 NNATNVVIKVCEFQFCNDPFLGVYYHKNNKSWMESEFRVYSSANNCTFEYVSQPFLMDLE
UW NNATNVVIKVCEFQFCNDPFLGVYYHKNNKSWMESEFRVYSSANNCTFEYVSQPFLMDLE
UC NNATNVVIKVCEFQFCNDPFLGVYYHKNNKSWMESEFRVYSSANNCTFEYVSQPFLMDLE
A1 NNATNVVIKVCEFQFCNDPFLGVYYHKNNKSWMESEFRVYSSANNCTFEYVSQPFLMDLE
T2 NNATNVVIKVCEFQFCNDPFLGVYYHKNNKSWMESEFRVYSSANNCTFEYVSQPFLMDLE
C1 NNATNVVIKVCEFQFCNDPFLGVYYHKNNKSWMESEFRVYSSANNCTFEYVSQPFLMDLE
C2 NNATNVVIKVCEFQFCNDPFLGVYYHKNNKSWMESEFRVYSSANNCTFEYVSQPFLMDLE
T1 NNATNVVIKVCEFQFCNDPFLGVYYHKNNKSWMESEFRVYSSANNCTFEYVSQPFLMDLE
K1 NNATNVVIKVCEFQFCNDPFLGVYYHKNNKSWMESEFRVYSSANNCTFEYVSQPFLMDLE

J1 GKQGNFKNLREFVFKNIDGYFKIYSKHTPINLVRDLPQGFSALEPLVDLPIGINITRFQT
J2 GKQGNFKNLREFVFKNIDGYFKIYSKHTPINLVRDLPQGFSALEPLVDLPIGINITRFQT
UW GKQGNFKNLREFVFKNIDGYFKIYSKHTPINLVRDLPQGFSALEPLVDLPIGINITRFQT
UC GKQGNFKNLREFVFKNIDGYFKIYSKHTPINLVRDLPQGFSALEPLVDLPIGINITRFQT
A1 GKQGNFKNLREFVFKNIDGYFKIYSKHTPINLVRDLPQGFSALEPLVDLPIGINITRFQT
T2 GKQGNFKNLREFVFKNIDGYFKIYSKHTPINLVRDLPQGFSALEPLVDLPIGINITRFQT
C1 GKQGNFKNLREFVFKNIDGYFKIYSKHTPINLVRDLPQGFSALEPLVDLPIGINITRFQT
C2 GKQGNFKNLREFVFKNIDGYFKIYSKHTPINLVRDLPQGFSALEPLVDLPIGINITRFQT
T1 GKQGNFKNLREFVFKNIDGYFKIYSKHTPINLVRDLPQGFSALEPLVDLPIGINITRFQT
K1 GKQGNFKNLREFVFKNIDGYFKIYSKHTPINLVRDLPQGFWALEPLVDLPIGINITRFQT

J1 LLALHRSYLTPGDSSSGWTAGAAAYYVGYLQPRTFLLKYNENGTITDAVDCALDPLSETK
J2 LLALHRSYLTPGDSSSGWTAGAAAYYVGYLQPRTFLLKYNENGTITDAVDCALDPLSETK
UW LLALHRSYLTPGDSSSGWTAGAAAYYVGYLQPRTFLLKYNENGTITDAVDCALDPLSETK
UC LLALHRSYLTPGDSSSGWTAGAAAYYVGYLQPRTFLLKYNENGTITDAVDCALDPLSETK
A1 LLALHRRYLTPGDSSSGWTAGAAAYYVGYLQPRTFLLKYNENGTITDAVDCALDPLSETK
T2 LLALHRSYLTPGDSSSGWTAGAAAYYVGYLQPRTFLLKYNENGTITDAVDCALDPLSETK
C1 LLALHRSYLTPGDSSSGWTAGAAAYYVGYLQPRTFLLKYNENGTITDAVDCALDPLSETK
C2 LLALHRSYLTPGDSSSGWTAGAAAYYVGYLQPRTFLLKYNENGTITDAVDCALDPLSETK
T1 LLALHRSYLTPGDSSSGWTAGAAAYYVGYLQPRTFLLKYNENGTITDAVDCALDPLSETK
K1 LLALHRSYLTPGDSSSGWTAGAAAYYVGYLQPRTFLLKYNENGTITDAVDCALDPLSETK

J1 CTLKSFTVEKGIYQTSNFRVQPTESIVRFPNITNLCPFGEVFNATRFASVYAWNRKRISN
J2 CTLKSFTVEKGIYQTSNFRVQPTESIVRFPNITNLCPFGEVFNATRFASVYAWNRKRISN
UW CTLKSFTVEKGIYQTSNFRVQPTESIVRFPNITNLCPFGEVFNATRFASVYAWNRKRISN
UC CTLKSFTVEKGIYQTSNFRVQPTESIVRFPNITNLCPFGEVFNATRFASVYAWNRKRISN
A1 CTLKSFTVEKGIYQTSNFRVQPTESIVRFPNITNLCPFGEVFNATRFASVYAWNRKRISN
T2 CTLKSFTVEKGIYQTSNFRVQPTESIVRFPNITNLCPFGEVFNATRFASVYAWNRKRISN
C1 CTLKSFTVEKGIYQTSNFRVQPTESIVRFPNITNLCPFGEVFNATRFASVYAWNRKRISN
C2 CTLKSFTVEKGIYQTSNFRVQPTESIVRFPNITNLCPFGEVFNATRFASVYAWNRKRISN
T1 CTLKSFTVEKGIYQTSNFRVQPTESIVRFPNITNLCPFGEVFNATRFASVYAWNRKRISN
K1 CTLKSFTVEKGIYQTSNFRVQPTESIVRFPNITNLCPFGEVFNATRFASVYAWNRKRISN
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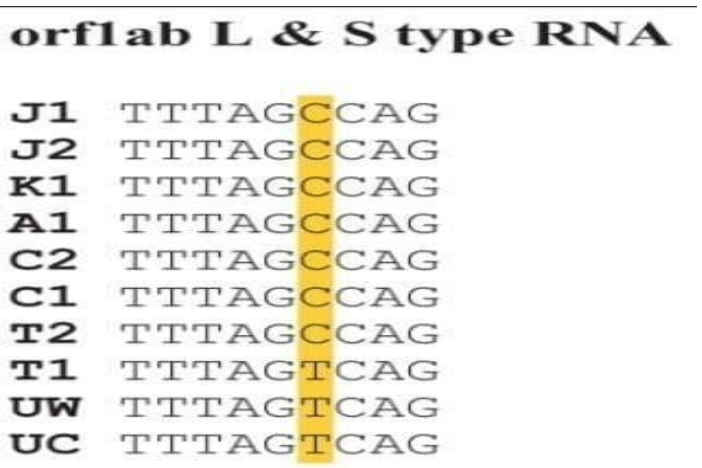
In s protein one amino acid mutation at "W" from South Korea comparing "S" in other nine sequences. One amino acid mutation at "R" from Australia was observed comparing "S" from another nine sequences. Two yellow lines indicate the difference in 10 sequence alignments.

Genomic analysis of ORF8 protein amino acid sequence. possible found in OFR8 with L and S subtypes



Genomic analysis of orf1ab protein amino acid sequence. subtypes were were found orf1b C and T.

Result:-

. E protein :-

E protein has a short and hydrophilic N-amino terminus consisting of 7–12 amino acids, followed by a large hydrophobic transmembrane domain of 25 amino acids, and ends with a long, hydrophilic C-carboxyl terminus and found one mutation in korea

S protein:-

S protein mediates attachment of SARS-CoV1 to the host cell surface receptors and facilitate viral entry into the host cell and by alignment found two mutation one in Australia and another in south korea

M and N proteins:-

The M protein is abundant which defines the shape of the viral envelope. N functions primarily to bind to RNA genome of SARS-Co2V, making up the nucleocapsid.15 Although N protein is most involved in processes viral genome signaling, it is also involved RNA replication cycle with host cellular response to viral infection ,by alignment not found any mutation in N and M protein

DISCUSSION:-

. point mutation:-

. we found point mutation in E protein and no mutation observed in M and N protein .in s protein found 2 mutation at “W” , “R” Report mentioned a single amino acid reversion (L294-to-Q) in the S protein is sufficient to abrogate the phenotype and grows well at and below 32ºC

. SNP or subtype:-

we found that SNPs at locations 8782 (orf1ab: T8517C, synonymous) and 28144 (ORF8: C251T, S84L) showed possible linkage in 10 sequences from different countries. As report “On the origin and continuing evolution of SARS-CoV-2” emphasized two subtypes of “L” and “S” from their data (defined as “L” type because T28144 is in the codon of leucine) and other “TC” haplotype (defined as “S” type because C28144 is in the codon of serine) at these two sites.

. conclusion:-

we analyzed 10 sequences from the NCBI data base by genome alignment and found no difference in amino acid sequences within M and N proteins. There are two amino acid variances in S protein region. One mutation found from South Korea sequence is verified. Two possible “L” and “S” SNPs found in ORF1ab and ORF8 regions are detected

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