Decoding the lethal effect of *SARS-CoV-2* (novel coronavirus) strains from global perspective: molecular pathogenesis and evolutionary divergence

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Abstract

Background COVID-19 is a disease with global public health emergency that have shook the world since its' first detection in China in December, 2019. Severe acute respiratory syndrome Coronavirus 2 (*SARS-CoV-2*) is the pathogen responsible behind this pandemic. The lethality of different viral strains is found to vary in different geographical locations but the molecular mechanism is yet to be known.

Methods Available data of whole genome sequencing of different viral strains published by different countries were retrieved and then analysed using Multiple Sequence Alignment and Pair-wise Sequence Alignment leading to Phylogenetic tree construction. Each location and the corresponding genetic variations were screened in depth. Then the variations are analysed at protein level giving special emphasis on Non Synonymous amino acid substitutions. The fatality rates in different countries were matched against the mutation number, rarity of the nucleotide alterations and functional impact of the Non Synonymous changes at protein level, separately and in combination.

Findings All the viral strains have been found to evolve from the viral strain of Taiwan (MT192759) which is 100% identical with the ancestor *SARS-CoV-2* sequences of Wuhan (NC 045512.2; submitted on 5th Jan, 2020). Transition from C to T (C>T) is the most frequent mutation in this viral genome and mutations A>T, G>A, T>A are the rarest ones, found in countries with maximum fatality rate i.e Italy, Spain and Sweden. 20 Non Synonymous mutations are located in viral genome spanning Orf1ab polyprotein, Surface glycoprotein, Nucleocapsid protein etc. The functional effect on the structure and function of the protein can favourably or unfavourably interact with the host body.

Interpretation The fatality outcome depends on three important factors (a) number of mutation (b) rarity of the allelic variation and (c) functional consequence of the mutation at protein level. The molecular divergence, evolved from the ancestral strain (S) lead to extremely lethal (E), lethal(L) and non lethal (N) strains with the involvement of an Intermediate strain(I).

Introduction

In the new decade of 21st century, surfaced the first public health emergency of global concern in Wuhan, China from the menace of COVID-19 (*nCoV/β-coronavirus*), also known as *SARS-CoV-2*. COVID-19 is one of the seven pathogenic members of family *coronaviridae*, other notable ones are severe acute respiratory syndrome (SARS) coronavirus (*SARS-CoV*), identified first in southern China (November, 2002), and Middle East respiratory syndrome (MERS) coronavirus (*MERS-CoV*), from Saudi Arabia (2012). Less severe ones are *HKU1*, *NL63*, *OC-43* and *229E*.[1] The enveloped virus possesses crown-like spikes on their surface (Latin word *corona* i.e crown) and a RNA genome, single stranded positive-sense strand with 26 to 32 kb length.[2] The microbe can co-infect different vertebrates including humans and affect organs of respiratory, gastrointestinal and central nervous system.

According to World Health Organization (WHO), 80% COVID infection are mild or asymptomatic, 15% are severe infection, while 5% are critical. Crude mortality ratio (no. of reported deaths divided by reported cases) is between 3-4%. The infection mortality rate (the number of reported deaths divided by the number of infection) will be lower than seasonal influenza (0·1%). Globally there are 6,68,690 confirmed cases; 31,065 deaths and so far 1,43,107 people recovered in 199 countries and territories.[3,4]

The most vulnerable group in corona epidemic is elderly, malnourished, hypertensive, diabetic, immune compromised, cancer and cardiovascular patients, as well as pregnant women. Despite wide spread COVID-19 infection, the numbers of fatally infected children cases are less reported so far, possible mechanism might be an unknown protective interaction between immune system and respiratory pathway.[5] COVID-19 spreads through droplet infection and fomites, from infected person during coughing and sneezing. Majority of symptoms are sore throat, breathing difficulty and fever, although GI and musculoskeletal system are also involved.[6,7] Clinical course is somehow showing a predictable pattern. On around day five, flu like symptom starts, common are fever, headache, dry cough, myalgia (and back pain), nausea (without vomiting), abdominal discomfort with some diarrhea, loss of smell, anorexia and fatigue. Around same time symptoms can worsen leading to shortness of breath due to bilateral viral pneumonia from direct viral damage to lung parenchyma. Around day10, cytokine storm kicks in, subsequently acute respiratory distress syndrome (ARDS) and multi organ failure ensues. This degradation usually happens in matter of hours. Hospitalized patients in moderate to severe cases usually come in hypoxic stage without dyspnea. China reported 15% cardiac involvement and serious final outcome. In most of the chest X-rays, bilateral interstitial pneumonia or ground glass opacities are seen. Hypoxia mostly does not correlate well with the chest x-ray findings. Chest auscultations are not of great help. Blood reports show, in the most cases, WBC is low, i.e. mostly lymphocytopenia, low platelet count whereas pro-calcitonin are mostly normal. Most consistently, CRP and ferritin levels are elevated and similarly CPK, D-dimer, LDH, ALK-phos/AST-ALT levels are also high. It has been seen a ratio of absolute neutrophil count to absolute lymphocyte count, greater than 3.5, may be the highest predictor of poor outcome. An elevated level of IL-6 is of grave concern of cytokine storm.[8,9]

Among the four classified subfamilies, α- and β-coronaviruses both were found in bats, e.g Leaf-nose bats like *Hipposideros armige* harbor *α-coronavirus* and *β-coronavirus* in *Hipposideros larvatus*;[10] while *γ-* and *δ-coronavirus* found in pigs and birds respectively.[11] The genesis of *β-coronavirus* cluster in afore mentioned bat species, consisting of primeval and genetically

independent types might took favour of host/pathogen interaction despite of wide geographical distribution.[10] Genomic characterization of *SARS-CoV-2* reveals 96% identity to that of bat coronavirus whole genome;[1] while 79% and 50% to that of *SARS-CoV* and MERS-CoV respectively.[12] Like other betacoronaviruses, the genome of *SARS-CoV-2* has a long ORF1ab polyprotein at the 5' end, followed by four major structural proteins, including the spike like surface glycoprotein, small envelope protein, matrix protein, and nucleocapsid protein.[11,13] The spike (S) protein has two distinct functional domains, termed S1 and S2, both of which are necessary for a coronavirus to successfully enter a cell. It has been found that S protein of *SARS-CoV-2* is 10-20 times more likely to bind to human ACE2 than the S protein of the early 2000s *SARS-CoV* strain. ACE2 is essentially a carboxypeptidase, which can remove carboxy-terminal of hydrophobic or basic amino acids. The expression of ACE2 is comparatively higher in mouth and tongue and is also normally expressed in human lower lungs on type-I and type-II alveolar epithelial cells. The heightened affinity for a prevalent cellular receptor may be a factor which increases a quick transmission of *SARS-CoV-2* in the upper respiratory tract. As an RNA virus, 2019-nCoV has the inherent feature of a high mutation rate. Due to mutations and recombination effects, different viral strains are originating with new characteristics; however because of its genome encoded exonuclease, the mutation rate might be somewhat lower than other RNA viruses.[12,14]

In this study, we comprehensively analyzed the whole genome sequence homology from the available patient data uploaded by affected countries in NCBI Virus database, identified the mutations developed by different strains from the ancestor strain and studied the impact of those mutations at functional level. Our endeavour was to categorize all the strains into major groups depending upon their lethal effect and mutations observed.

Methodology

Whole genomic data retrieval from the database

Retrieved the whole genome sequences from "NCBI Virus" database, specific input was "*SARS-CoV-2*", during a period from Jan 5 through March 24, 2020. Later submissions or the sequences with many undetermined nucleotides (denoted as N) were not considered.[15] Countries those had multiple entries, highest number of entries with whole genome sequence were matched for identifying representative sequence to consider (e.g. for USA, out of 44 submitted complete genomic sequences, the sequence with 29 submissions were of complete genomic sequence with 100% identity and thus one representative sequence was considered for the present study). Thus, 13 complete whole genome sequences submitted by 13 different affected countries were taken into consideration (figure 1).

Multiple Sequence Alignment and Evolutionary Dynamics

ClustalW (version 2.1) was employed to align multiple sequences, representative of 13 countries, with the purpose (a) to understand the similarity and variation in all genomic position, (b) to establish the evolutionary relationship among the viral strains affecting the whole world and (c) finally, to distinguish the ancestry of different viral strains using FastTree tool, which is based on Neighbour joining method.

Pair-wise Sequence Alignment

EMBOSS-Stretcher was used for whole genomic pair-wise alignment between the ancestor *SARS-CoV-2* sequences of Wuhan (NC045512.2; submitted on 5th Jan, 2020) with all 13 sequences. Similar strategy was used for pair wise alignment between the genome sequences of *SARS-CoV* submitted by Italy, 2003 with the *SARS-CoV-2* strain of Italy, 2020.

Screening of Mutations

All alignment results were thoroughly screened to find out the exact location of the mutations and the genetic variation affecting that position. Functional implications of each type of nucleotide variations were understood from the LODS ratio and it is calculated by the formulae $\log_e$ (Obs/Exp), where 'Obs' is the observed frequency of a specific alteration in the genome and 'Exp' is the expected frequency of that specific alteration due to individual proportion of the nucleotides in the genome sequences.

Variation analysis at Protein level

To obtain the different protein sequences of the translated genome, open reading frame was generated using ORF Finder. Six different ORF were generated which covered the entire genome sequence. Our protein sequences were aligned with existing protein sequences of *SARS-CoV-2* present in the 'NCBI Virus' database to check which ORF corresponds to the which known protein. The amino acid alterations were identified from the nucleotide information and were classified into Synonymous (S) and Non Synonymous (NS). The functional impacts of all NS mutations at protein were analysed using different SNP annotation tools (i.e. SIFT, SNAP, Polyphen2 and MetaSNP).

Fatality rate and underlying molecular predisposition

The fatality rate of a country was calculated considering the summation of total number of death and critical case and then dividing the same with total number of detected case. [Fatality rate = (Total no. of deaths + total no of Severe cases)/ Total no. of detected cases]). The fatality rates were matched against the rarity of the nucleotide alterations and functional impact of the Non-synonymous changes at protein level.

Origin and evolutionary explanations of *SARS-CoV-2* strains

All the 13 Strains of 13 different geographical locations were categorized depending upon the no of mutations and type of mutations they had.

Results

MSA of whole genome sequences and identified mutations

Multiple sequence alignment of 13 whole genome sequences, representative entries from 13 countries, clearly showed extremely conserved sequence with only 35 mutations (Supplementary Material 1). Only viral genome sequence identified from Taiwan entry does not show any mutation, suggesting this is the ancestral genome sequence. All the mutations of *SARS-CoV-2*

identified were reported in Table 1. Among all mutations, a three nucleotide deletion (from 21964-21966 position) was observed only from MT012098 strain of India.

Phylogenetic tree and evolutionary dynamics

The phylogenetic tree analysis showed the proximity of the viral strains of different COVID-19 affecting countries. Three distinct clusters have been found (figure 2) - one comprises of Sweden (MT093571), Australia (MT007544), Italy (MT066156) and Brazil (MT126808); second one comprises of Finland (MT020781), USA (MT027064) and the 3rd cluster comprises of China (MT135043) and Spain (MT233523). MT192759 strain of Taiwan is at middle position of the phylogram depicting that all the strains have diverged from it through mutations. MSA results also depicted it to be the ancestor strain of *SARS-CoV-2* with zero mutation.

Pair-wise Alignment between the ancestor viral strains of Wuhan with all 13 sequences

The first submitted Ancestor viral Strain of Wuhan (Submitted 5th Jan, 2020; NC_045512·2) showed 100% identity with the viral Strain of Taiwan (MT192759·1) only and confirmed our previous observation (Supplementary Material 2). The comparison between *SARS-CoV* and *SARS-CoV-2* Strain showed approximately 79.2% identity; and that of *SARS-CoV* (AY323977, 2003 strain) and *SARS-CoV-2* (MT066156, 2020 strain) of Italy found 11 out of 35 mutations to be common, suggesting viral strain of Wuhan and Taiwan are identical and *SARS-CoV* shares common features with different strains of *SARS-CoV-2* (Supplementary Material 3).

Screening of Mutations

All the mutations are screened depending upon their rarity to occur in this viral genome and A>T, G>A and T>A are found to be very rare alterations with LOD score value less than -0·5 where as C>T is the most common alteration with LOD score 1·81.

Variation analysis at Protein level

Different protein sequences are obtained using ORF finder and those sequences were matched with existing Orf1ab polyprotein, spike like surface glycoprotein, nucleocapsid phosphoprotein etc. to ensure prediction of *in silico* ORF with corresponding *SARS-CoV-2* protein. Next, observed mutations were confirmed at amino acid level and identified a total of 20 Non-synonymous (NS) and 14 Synonymous (S) mutations (Table 2). The analysis with different SNP annotation tools like SIFT,[16] SNAP,[17] Polyphen2,[18] MetaSNP[19] showed the functional impact of NS mutations at different level, predicting either tolerance of the mutation, or disease causing ability, or executing risk or damage, which altogether can infer the fatality rate.

Fatality Rate Calculation and underlying molecular predisposition

The fatality rate of Italy (14·82%), Spain (13·77%) and Sweden (9·20%) were significantly higher, where it was less than 4% in countries like Nepal (Zero fatality), Finland (2·63%), Vietnam (2·01%), USA (3·80), Australia (0·91%) and India (2·06). In case of Japan, Brazil and China, fatality rate was moderate i.e approximately 4-8% (figure 3). The number of mutations and presence of either of the very rare mutations or functionally important NS mutations or both are found to be strongly linked with the fatality rate (Table 3A-Table 3C).

Origin and evolution of *SARS-CoV-2* strains

It is extremely important to understand how the ancestral strain (S) leads to lethal strain (L) and other clusters observed according to phylogeny. Our analysis showed Ancestral strain (S) give rise to an Intermediate strain (I) with single very common mutation, a transition from C to T. From I, three different strains originate, i.e (a) Lethal strain (L), with additional mutations over I, (b) Extremely lethal strain (E) that contains very rare mutations and a (c) Non Lethal strain (N) that contains favourable mutations at surface glycoprotein, which possibly inhibit the interaction of ACE2 and favouring non-lethal outcome (figure 4).

Discussion

The current global pandemic of the novel coronavirus Covid-19 created two epicenters first at Hubei Province in People's Republic of China and presently in Europe. *SARS-CoV-2*, like its close relatives *SARS-CoV* and *MERS-CoV*, is also pathogenic but with a higher infectivity rate. The increasing number of cases and wide spread disease raise grave concerns about the future trajectory of the pandemic and thus, a better understanding regarding the molecular divergence of viral strains and pathogenesis, is utmost important.

The present study attempted to categorize COVID affected countries based on molecular pathogenesis. Three important factors were considered, i.e, number of mutations during evolution, rarity of the allelic substitution and functional alteration of the non-synonymous mutations. We screened and compared extent of mutations observed in genome sequence of *SARS-CoV-2*. All reported genome sequences of 13 affected countries have been analyzed. So far, studies indicated that there are only two strains, S, possibly the ancestral one and L, might be the lethal and more aggressive one. The distinct clusters that we observed from our phylogenetic tree construction raise the assumption of existence of many more unknown strains (figure 5). One interesting report substantiating our finding is from Shenzhen which showed possible generation of new strain which neither belongs to 'S' nor to 'L' subtype.[12] We evaluated the extent of molecular divergence by MSA and Pair-wise alignment and subsequently tried to decipher the protein alteration parallel to the mutations.

As a RNA virus, it was hypothesized that 2019-nCoV mutate faster than DNA virus.[12] The genome-wide phylogenetic tree indicated that 2019-nCoV was closest to SARS-like coronavirus, with 96% sequence similarity.[20,21] When we evaluated the *SARS-CoV* (2003) and *SARS-CoV-2* (2020) from Italy, sequence identity was 79.4%, suggesting new mutations, might be deleterious in later strain.

Our first approach was to categorize the countries depending on number of mutations and type of mutation they had. It was found that similar number of mutations does not correlate well with similar extent of fatality outcome. Example, patients from Australia, Japan and Italy all show 3 mutations in the viral genome, but Australia has least fatality rate, while Italy has maximum. Again, numbers of mutations are 5 in China, 6 in India and 7 in both Sweden and Spain. The observations mentioned above strongly indicate that not the total number of mutation, but the nature of mutations finally guides the overall fatality outcome. In the case of Italy, we found three deadly mutations in 2020 (i.e. 2269 A>T, very rare allele; 11083 G>A, very rare allele and

26144 G>T) with high disease outcome but the wild counterpart of these found in 2003 did not lead to any fatality, suggesting the significance of newly evolved mutations. Although, 2269 A>T mutation does not alter amino acid (A668A), increasing number of evidences suggest that synonymous mutations could have effects on splicing, transcription, that ultimately alter the phenotype, disrupting their silence.[22] Spain is also carrying a very rare allele transversion T>A, that occurs in orf1ab gene of virus. Orf1ab gene transcribes into a polyprotein and cleaving by protease (3CLpro) and papain-like protease (PLpro) produces several non-structural proteins, which are important for replication as well as virulence for coronavirus. Thus, an alteration in this region might alter the virulence, and associated fatality outcome.

We assume, while some mutations are pathogenic, some will be favorable and will undergo positive selection pressure. Herein, we tried to elucidate the possible interaction between ACE2 and spike glycoprotein. It is well established that for virus entry, spike glycoprotein (S) present on the CoV can be a neutralization antibody and it binds to its receptor followed by membrane fusion. It has been inferred that some favorable changes in viral glycoprotein may limit the increase of fatality. This kind of favorable mutation was found in some strains of COVID-19 from India, Australia and Sweden. Detailed screening depicted that Australian strain carry a rare allele transversion (T>G) which results into a NS mutation (S256R) on surface glycoprotein S1 domain which may affect the binding of hACE2 molecules, thus these strains become non-lethal to human. In this study, only one tri-nucleotide deletion at S1 domain of surface glycoprotein has been found and that is found in Indian strains along with a NS mutation (R417I) in the Receptor Binding Domain (RBD) of S1 subunit that disfavor viral entry by inhibiting hACE2 interaction.

On the other hand, Brazilian strain acquires four common allelic transitions, of which only two non-synonymous mutations affect protein alteration. Surprisingly, Swedish strain carry both extreme lethal (2717 G>A, very rare allele leading to G818S at Orf1ab polyprotein) and also favorable mutations (23952 T>G, rare allele leading to F806C at surface glycoprotein) and thus cumulatively lower the severity of the disease.

In summary, the present study reveals that the fatality rate increases with not only the number of mutations but also depending on its allelic rarity as well as functional alteration of protein. Surface glycoprotein domain is very important for host-carrier interactions and hence the mutations affecting surface glycoprotein can be one of the important mechanisms which alter the viral entry and pathogenesis. Future studies may uncover more genetic information at the molecular level as well as structural levels of the proteins, because without this knowledge it'll be difficult to identify drug target and prepare vaccine. We hope our work will help in that direction.

Author Contribution

SB, PB designed the study; SB, SD analyzed the data; SB, PB interpreted the data; SB, SD, SBh searched literatures; SD, SBh, PB wrote the manuscript; SD prepared figures; PB supervised overall study.

Declaration of Interest

We declare no competing interests.

Acknowledgement

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Table 1: Database of Single Nucleotide Polymorphisms found by whole genome analysis of different strains of *SARS-CoV-2* retrieved from 'NCBI virus' database and their rarity in *SARS-CoV-2* genome

SNP No	SNP Position	Wild Type Residue	Mutated Residue	Conservation Probability of wild type	Variation type	Expected Frequency	Observed Frequency	LODS Ratio	Rarity in the genome
1	2091	C	T	0·923	C>T	0·0615	0·375	1·81	Very Common
2	2269	A	T	0·923	A>T	0·0874	0·05	-0·56	Very Rare
3	2277	T	C	0·923	T>C	0·0615	0·175	1·05	Common
4	2717	G	A	0·923	G>A	0·0592	0·025	-0·86	Very Rare
5	4402	T	C	0·923	T>C	0·0615	0·175	1·05	Common
6	5062	G	T	0·923	G>T	0·0641	0·20	1·14	Common
7	6695	C	T	0·923	C>T	0·0615	0·375	1·81	Very Common
8	8782	C	T	0·923	C>T	0·0615	0·375	1·81	Very Common
9	9274	A	G	0·923	A>G	0·0592	0·05	-0·16	Rare
10	9477	T	A	0·923	T>A	0·0874	0·025	-1·25	Very Rare
11	10232	C	T	0·923	C>T	0·0615	0·375	1·81	Very Common
12	11083	G	T/N	0·846	G>T	0·0641	0·20	1·14	Common
					G>A	0·0592	0·025	-0·86	Very Rare
13	13225	C	G	0·923	C>G	0·0416	0·05	0·18	Common
14	13226	T	C	0·923	T>C	0·0615	0·175	1·05	Common
15	14657	C	T	0·923	C>T	0·0615	0·375	1·81	Very Common
16	14805	C	T	0·846	C>T	0·0615	0·375	1·81	Very Common
17	15324	C	T	0·923	C>T	0·0615	0·375	1·81	Very Common
18	17247	C	T	0·923	C>T	0·0615	0·375	1·81	Very Common
19	17373	C	T	0·923	C>T	0·0615	0·375	1·81	Very Common
20	17376	A	G	0·923	A>G	0·0592	0·05	-0·16	Rare
21	19065	T	C	0·923	T>C	0·0615	0·175	1·05	Common
22	21707	C	T	0·846	C>T	0·0615	0·375	1·81	Very

									Common
23	21964-66	TTA	---	0·923	NA	NA	NA	NA	NA
24	22303	T	G	0·923	T>G	0·0641	0·05	-0·24	Rare
25	22785	G	T	0·923	G>T	0·0641	0·20	1·14	Common
26	23952	T	G	0·923	T>G	0·0641	NA	NA	NA
27	24034	C	T	0·923	C>T	0·0615	0·375	1·81	Very Common
28	25810	C	G	0·923	C>G	0·0416	0·05	0·18	Common
29	25979	G	T	0·923	G>T	0·0641	0·20	1·14	Common
30	26144	G	T	0·692	G>T	0·0641	0·20	1·14	Common
31	28144	T	C	0·846	T>C	0·0615	0·175	1·05	Common
32	28657	C	T	0·923	C>T	0·0615	0·375	1·81	Very Common
33	28863	C	T	0·923	C>T	0·0615	0·375	1·81	Very Common
34	29301	A	T	0·923	A>T	0·0874	0·05	-0·56	Very Rare
35	29303	C	T	0·923	C>T	0·0615	0·375	1·81	Very Common

Table 2: Effect of mutation in protein level and Fatality rate in different geographical location (Shown in ascending order of achieving mutation by the strain)

Viral Strain (Accession No)	Country Name	Total no of Mutation	Mutation position	Variation type	Codon Alteration	Effect on Protein Sequence	Mutation Catagory	Fatality Rate
MT192759	Taiwan	0	NA	NA	NA	NA		0·80
MT072688	Nepal	1	24034	C>T	AAC>AAT	N833N	S	0·00
MT020781	Finland	1	21707	C>T	CAT>TAT	H58Y	NS	2·63
MT192772	Vietnam	1	10232	C>T	CGT>TGT	R3323C	NS	2·01
MT027064	USA	2	2091	C>T	ACT>ATT	T609I	NS	3·80
			21707	C>T	CAT>TAT	H58Y	NS	
MT007544	Australia	3	19065	T>C	CCT>CCC	P1766P	S	0·91
			22303	T>G	AGT>AGG	S256R	NS	
			26144	G>T	GGT>GTT	G251V	NS	
LC529905	Japan	3	15324	C>T	AAC>AAT	N519N	S	7·57
			25810	C>G	CTT>GTT	L140V	NS	
			29303	C>T	CCA>TCA	P344S	NS	
MT066156	Italy	3	2269	A>T	GCA>GCT	A668A	S	14·82
			11083	G>N	TTG>TTT/A	L3606F	NS	
			26144	G>T	GGT>GTT	G251V	NS	
MT126808	Brazil	4	11083	G>T	TTG>TTT	L3606F	NS	7·33
			14805	C>T	TAC>TAT	Y346Y	S	
			17247	T>C	CGT>CGC	R1160R	S	
			26144	G>T	GGT>GTT	G251V	NS	
MT135043	China	5	4402	T>C	CTT>CTC	L1379L	S	5·45
			5062	G>T	TTG>TTT	L1599F	NS	
			8782	C>T	AGC>AGT	S2839S	S	
			28144	T>C	TTT>TTC	F87F	S	
			29301	A>T	GAT>GTT	D343V	NS	
MT012098	India	6 (includes a deletion)	2277	T>C	ATT>ACT	I671T	NS	2·06
			6695	C>T	CCT>TCT	P2144S	NS	
			14657	C>T	GCT>GTT	A306V	NS	
			17373	C>T	GCC>GCT	A1202A	S	
			22785	G>T	AGA>ATA	R417I	NS	
			21964-66	TTA Deletion	TATTAC>TAC	Y154 Deletion	Deletion	
MT093571	Sweden	7	2717	G>A	GGT>AGT	G818S	NS	9·20
			9274	A>G	AGA>AGG	R3003R	S	
			13225	C>G	TCC>TCG	S4320S	S	
			13226	T>C	TTT>CTT	F4321L	NS	
			17376	A>G	ACA>ACG	T1203T	S	
			23952	T>G	TTT>TGT	F806C	NS	
			26144	G>T	GGT>GTT	G251V	NS	

MT233523	Spain	7	8782	C>T	AGC>AGT	S2839S	S	13·77
			9477	T>A	TTT>TAT	F3071Y	NS	
			14805	C>T	TAC>TAT	Y346Y	S	
			25979	G>T	GGA>GTA	G196V	NS	
			28144	T>C	TTT>TTC	F87F	S	
			28657	C>T	GAC>GAT	D128D	S	
			28863	C>T	TCA>TTA	S197L	NS	

S = Synonymous Mutation, NS = Non Synonymous Mutation

Table 3A: Countries with Fatality Rate <4% and underlying molecular predisposition

Country Name	Mutation position	Nucleotide Alteration	Rarity of alteration in the genome	Effect on Protein Sequence	Annotation Tools				Impact of the amino acid alteration[#]	Gene	Nature of Protein
					SIFT Prediction	SNAP Prediction	MetaSNP Prediction	Polyphen 2 Prediction			
Taiwan	NA	NA		NA	NA	NA	NA	NA	NA	NA	NA
Nepal	24034	C>T	Very Common	N833N	NA	NA	NA	NA	Null	S	Surface Glycoprotein
Finland	21707	C>T	Very Common	H58Y	T	N	N	B	Very Low	S	Surface Glycoprotein
Vietnam	10232	C>T	Very Common	R3323C	NT	D	D	PD	Very High	Orf1ab	Orf1ab polyprotein
USA	2091	C>T	Very Common	T609I	T	N	D	B	Low	Orf1ab	Orf1ab polyprotein
	21707	C>T	Very Common	H58Y	T	N	N	B	Very Low	S	Surface Glycoprotein
Australia	19065	T>C	Common	P1766P	NA	NA	NA	NA	Null	Orf1ab	Orf1ab polyprotein
	22303	T>G	Rare	S256R	NT	D	N	B	Moderate	S	Surface Glycoprotein
	26144	G>T	Common	G251V	NT	D	D	PD	Very High	ORF3a	ORF3a protein
India	2277	T>C	Common	I671T	NT	D	N	B	Moderate	Orf1ab	Orf1ab polyprotein
	6695	C>T	Very Common	P2144S	T	N	N	PD	Low	Orf1ab	Orf1ab polyprotein
	14657	C>T	Very	A297V	NT	D	N	B	Moderate	Orf1ab	Orf1ab

		Common								polyprotein
17373	C>T	Very Common	A1202A	NA	NA	NA	NA	Null	Orf1ab	Orf1ab polyprotein
22785	G>T	Common	R417I	T	D	N	PD	Moderate	S	Surface Glycoprotein
21964-66	TTA Deletion		Y154 Deletion						S	Surface Glycoprotein

Impact of the amino acid alteration decoded based on: Synonymous Mutation = Null, Impact of amino acid variation; NA=Very Low (no predicting damage), Low (either of the 4 software prediction), Moderate (from any 2 software prediction), High (from 3 software prediction), Very High (all of the 4)

T = Tolerated, NT = Not Tolerated, N =Neutral, D =Damaging, B = Benign, Po = Possibly Damaging, PD = Probably Damaging

Table 3B: Countries with Fatality Rate 4-8% and underlying molecular predisposition

Country Name	Mutation position	Nucleotide Alteration	Rarity of alteration in the genome	Effect on Protein Sequence	SIFT Prediction	SNAP Prediction	MetaSNP Prediction	Polyphen 2 Prediction	Impact of the amino acid alteration[#]	Gene	Nature of Protein
Japan	15324	C>T	Very Common	N519N	NA	NA	NA	NA	Null	Orf1ab	Orf1ab polyprotein
	25810	C>G	Common	L140V	NT	N	N	Po	Moderate	ORF3a	ORF3a protein
	29303	C>T	Very Common	P344S	T	N	N	PD	Low	N	Nucleocapsid Phospho protein
Brazil	11083	G>T	Common	L3606F	NT	D	N	Po	High	Orf1ab	Orf1ab polyprotein
	14805	C>T	Very Common	Y346Y	NA	NA	NA	NA	Null	Orf1ab	Orf1ab polyprotein
	17247	T>C	Common	R1160R	NA	NA	NA	NA	Null	Orf1ab	Orf1ab polyprotein
	26144	G>T	Common	G251V	NT	D	D	PD	Very High	ORF3a	ORF3a protein
China	4402	T>C	Common	L1379L	NA	NA	NA	NA	Null	Orf1ab	Orf1ab polyprotein
	5062	G>T	Common	L1599F	T	N	N	Po	Low	Orf1ab	Orf1ab polyprotein
	8782	C>T	Very Common	S2839S	NA	NA	NA	NA	Null	Orf1ab	Orf1ab polyprotein
	28144	T>C	Common	F87F	NA	NA	NA	NA	Null	ORF8	ORF8 protein
	29301	A>T	Very Rare	D343V	NT	D	N	PD	High	N	Nucleocapsid Phospho protein

\# Impact of the amino acid alteration decoded based on: Synonymous Mutation = Null, Impact of amino acid variation; NA=Very Low (no predicting damage), Low (either of the 4 software prediction), Moderate (from any 2 software prediction), High (from 3 software prediction), Very High (all of the 4)

T = Tolerated, NT = Not Tolerated, N =Neutral, D =Damaging, B = Benign, Po = Possibly Damaging, PD = Probably Damaging
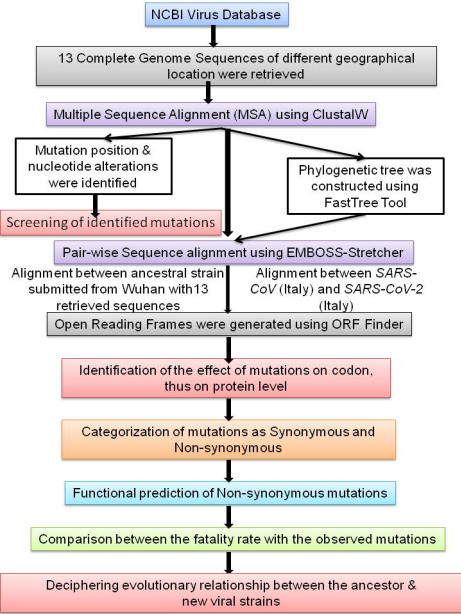
Table 3C: Countries with Fatality Rate >8 % and underlying molecular predisposition
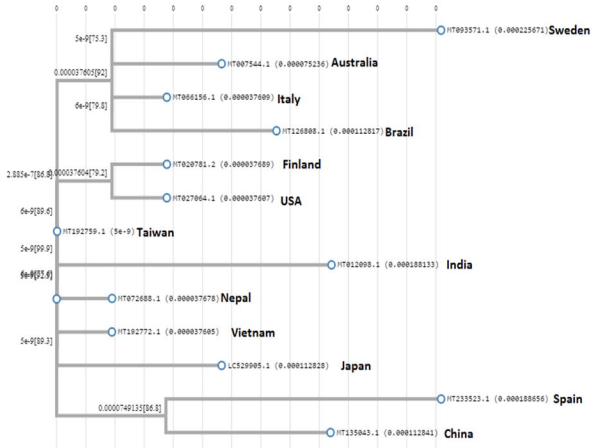
Country Name	Mutation position	Nucleotide Alteration	Rarity of alteration in the genome	Effect on Protein Sequence	SIFT Prediction	SNAP Prediction	MetaSNP Prediction	Polyphen2 Prediction	Impact of the amino acid alteration[#]	Gene	Nature of Protein
Italy	2269	A>T	Very Rare	A668A	NA	NA	NA	NA	Null	Orf1ab	Orf1ab polyprotein
	11083* (G>N)	G>T	Common	L3606F	NT	D	N	Po	High	Orf1ab	Orf1ab polyprotein
		G>A	Very Rare	L3606L	NA	NA	NA	NA	Null		
	26144	G>T	Common	G251V	NT	D	D	PD	Very High	ORF3a	ORF3a protein
Spain	8782	C>T	Common	S2839S	NA	NA	NA	NA	Null	Orf1ab	Orf1ab polyprotein
	9477	T>A	Very Rare	F3071Y	NT	D	D	PD	Very High	Orf1ab	Orf1ab polyprotein
	14805	C>T	Very Common	Y346Y	NA	NA	NA	NA	Null	Orf1ab	Orf1ab polyprotein
	25979	G>T	Common	G196V	NT	D	D	PD	Very High	ORF3a	ORF3a protein
	28144	T>C	Common	F87F	NA	NA	NA	NA	Null	ORF8	ORF8 protein
	28657	C>T	Very Common	D128D	NA	NA	NA	NA	Null	N	Nucleocapsid Phospho protein
	28863	C>T	Very Common	S197L	T	N	N	PD	Low	N	Nucleocapsid Phospho protein

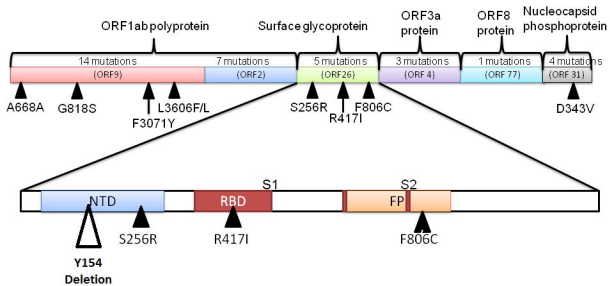|											
	2717	G>A	Very Rare	G818S	T	D	N	PD	Moderate	Orf1ab	Orf1ab polyprotein
	9274	A>G	Rare	R3003R	NA	NA	NA	NA	Null	Orf1ab	Orf1ab polyprotein
	13225	C>G	Common	S4320S	NA	NA	NA	NA	Null	Orf1ab	Orf1ab polyprotein
Sweden	13226	T>C	Common	F4321L	NT	D	N	PD	High	Orf1ab	Orf1ab polyprotein
	17376	A>G	Rare	T1203T	NA	NA	NA	NA	Null	Orf1ab	Orf1ab polyprotein
	23952	T>G	Rare	F806C	T	D	D	PD	High	S	Surface Glycoprotein
	26144	G>T	Common	G251V	NT	D	D	PD	Very High	ORF3a	ORF3a protein

Impact of the amino acid alteration decoded based on: Synonymous Mutation = Null, Impact of amino acid variationNA Very Low (no predicting damage), Low (either of the 4 software prediction), Moderate (from any 2 software prediction), High (from 3 software prediction),Very High (all of the 4)

T = Tolerated, NT = Not Tolerated, N =Neutral, D =Damaging, B = Benign, Po = Possibly Damaging, PD = Probably Damaging

FR= 5.45%, Mutation No.=5

FR= 2.63%, Mutation No.=1

FR= 7.57%, Mutation No.=3

FR= 3.80%, Mutation No.= 2

FR= 9.20%, Mutation No.=7

United States of America

China

Japan

FR= 13.77%, Mutation No.=7

India

Taiwan

FR= 0.00%, Mutation No.=1

FR= 14.82%, Mutation No.=3

FR= 0.80%, Mutation No.=0

FR= 7.33%, Mutation No.=4

Brazil

FR= 2.06%, Mutation No.=6

FR= 2.01%, Mutation No.=1

Australia

FR= 0.91%, Mutation No.=3
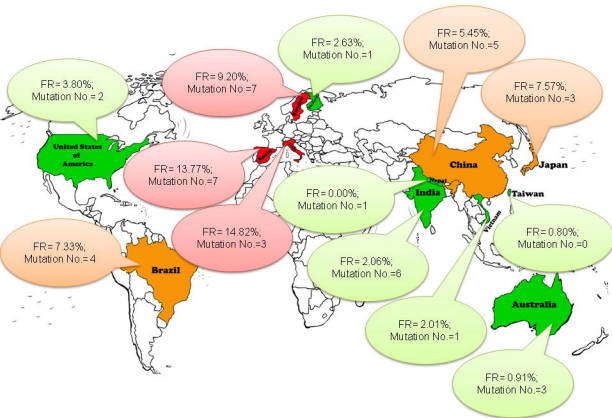
Ancestor strain (S)
NC045512 of Wuhan/MT192759 of Taiwan

Intermediate strain (I)
With single very common mutation (C>T)
MT192772 of Vietnam/MT020781 of Finland/MT072688 of Nepal/MT192765 of USA

Extremely Lethal strain (E)
harboring very rare mutations
MT066156 of Italy/MT233523 of
Spain/MT093571 of Sweden

Non Lethal strain (N)
harboring favorable mutation
at viral Surface glycoprotein
MT007544 of Australia/MT012098 of
India/ MT093571 of Sweden

Lethal strain (L)
harboring additional mutation over I
LC529905 of Japan/MT126808 of Brazil/MT135043 of China

Note: MT093571 of Sweden carries both very rare and favorable mutation, and thus probably lethality of that
strain is much lower to other two extremely lethal strain