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Geographical distribution of host’s specific SARS-CoV-2 mutations in the early phase of the COVID-19 pandemic

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ABSTRACT

Purpose: To assess, if the SARS-CoV-2 mutate in a similar pattern globally or has a specific pattern in any given population.

Results: We report, the insertion of TTT at 11085, which adds an extra amino acid, F to the NSP6 at amino acid position 38. The highest occurrence of TTT insertion at 11,085 position was found in UK derived samples (65.97%). The second and third highest occurrence of the mutation were found in Australia (8.3%) and USA (4.16%) derived samples, respectively.

Another important discovery of this study is the C27945T mutation, which translates into the termination of ORF-8 after 17 amino acids, reveals that the SARS-CoV-2 can replicate without the intact ORF-8 protein. We found that the 97% of C27945T mutation of global occurrence, occurred in Europe and the USA derived samples.

Conclusions: Two of the reported mutations (11085TTT insertion and C27945T nonsense), which seemed to reduce Type I interferon response are linked to specific geographical locations of the host and implicate region-specific mutations in the virus. The findings of this study signify that SARS-CoV-2 has the potential to adapt differently to different populations.

1. Introduction

The coronaviruses belong to the order Nidovirales, family Coronaviridae, and subfamily Coronavirinae (Cui et al., 2019). The COVID-19 pandemic is caused by the novel coronavirus, named as 2019-nCoV or SARS-CoV-2, belonging to the Betacoronavirus genus of the subfamily Coronavirinae. The SARS-CoV-2 virus contains a positive-sense single-stranded RNA (+ssRNA) genome with a size of around 29.9 kilobases (Chan et al., 2020). The genomes of coronaviruses are among the longest RNA viruses (Gorbalenya et al., 2006). Although coronavirus’ RNA-dependent RNA polymerase (RdRp) have a proofreading function, single-stranded RNA genomes have a higher mutation rate than double-stranded DNA viruses (Peck and Lauring, 2018 May 2). Another cause of mutation of the viral genome is the driving force to evade the host immune system defences. These factors make mutant variants of various viral proteins, which help the virus in evading the defences. Consequently, the fitting variants prevail in any geographical region/population over time.

Mutational changes in the viral genome may lead to sequence changes in untranslated regions (UTRs) as well as changes in amino acid sequences of structural and functional proteins of the viruses. Being the RNA virus, the SARS-CoV-2 is mutating; several rare and common mutations in UTR/ORFs in the genome have been already reported (Phan, 2020 Jul; Tang et al., 2020 Jun 1; Wang et al., 2020; Stefanelli et al., 2020; Kim et al., 2020; Pachetti et al., 2020 Dec 22). The D614G mutation in spike protein has got special attention among the SARS-CoV-2 virologist for becoming dominant around the world (Korber et al., 2020) and the mutation also correlated with a higher mortality rate in COVID-19 patients (Toyoshima et al., 2020 Dec 22). It is very likely that the SARS-CoV-2 may have more mutations like D614G, which may affect

Abbreviations: GISAID, Global initiative on sharing all influenza data; IRF3, Interferon regulatory factor 3; NCBI, National Center for Biotechnology Information; NSP6, Non-structural protein 6; ORF-8, Open Reading Frame-8; RdRp, RNA dependent RNA polymerase; TBK1, TANK binding kinase 1; UTRs, Untranslated regions.

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the viral infectivity and clinical outcome of the infection and hence, this study aims to explore such mutations more thoroughly.

Our aim was to analyse the SARS-CoV-2 genome sequences derived from patients of all six continents and to investigate whether the virus mutate in similar pattern globally or has specific pattern in any given populations or hosts. In the present, study we analysed 15,120 full length SARS-CoV-2 genomes, derived from symptomatic/asymptomatic COVID-19 patients from all six continents, submitted to NCBI Database (https://www.ncbi.nlm.nih.gov). We further investigated an additional 1,000 SARS-CoV-2 genome sequences, derived from Australia, China, India, Saudi Arabia (KSA), Egypt, Italy, Spain, USA, Mexico and Brazil submitted to GISAID (https://www.gisaid.org).

2. Methods

2.1. SARS-CoV-2 genome sequences

We analysed 15,120 full-length SARS-CoV-2 genomes (global set), derived from symptomatic/asymptomatic COVID-19 patients. These sequences were submitted to NCBI database from all six continents. We downloaded all the SARS-CoV-2 genome sequences available from the NCBI database as on June 25, 2020.

We also investigated another 1,000 SARS-CoV-2 genome sequences (country set) from symptomatic/asymptomatic COVID-19 patients from 10 countries (Australia, China, India, KSA, Egypt, Italy, Spain, USA, Mexico and Brazil, covering all six continents) as large number of SARS-CoV-2 infection cases have been reported from these countries. The sequences were downloaded from GISAID and 100 full-length SARS-CoV-2 genome sequences were randomly selected from each country.

2.2. Mutational analysis of the SARS-CoV-2 genome sequences

The 15,120 full-length SARS-CoV-2 genome sequences were processed using FASTA_Unequal_Sequences_1.0 tool (https://www.ncbi). Next, we used a sequencing tool, Minimap2 (Li, 2018 Sep 15), which is designed for long-read sequences with a larger number of samples to be aligned. Using Minimap2, once aligned with a reference sequence (accession number NC_045512.2) (WuF and YuB., 2020), the duplicate sequences were removed more aggressively and that gave us 400 genome sequences with mutations (Workflow: Step A). Although the MiniMap2 is suited for longer sequences and large number of samples, it has the disadvantage of showing the most common mutations only and hence we had to utilize another approach to find infrequent mutations.

To analyse the 1000 full-length SARS-CoV-2 genome sequences, derived from the aforementioned countries, we have designed a software tool, for fragmentation of nucleotide sequences. This tool helped us to fragment the SARS-CoV-2 genome sequences into 5000 base sections (Khalid et al., 2022). We have used MultAlin online tool (http://multalin) for alignment of fragmented genome sequences with the reference viral strain sequence mentioned above. We have taken care of not to miss part of the genome sequences by having 100 bases long overlapping sequences in each fragment. We considered variation as true mutation if variations from reference sequence were identified in more than one instances. The same practice was applied with the genome sequence samples of all six countries (Workflow: Step B). The results were validated by the MiniMap2 tool as well as with published data.

2.3. The occurrence of 11085TTT insertion and C27945T mutations in the SARS-CoV-2 genome

We downloaded all the SARS-CoV-2 genome sequences from GISAID database available on September 2, 2020, that provided us 93,265 genome sequences. We focussed our analysis for occurrence of 11085TTT insertion that translate to Phenylalanine (F) amino acid at the position 38 in non-structural protein 6 (NSP6). Additionally, C27945T mutation, which translates to stop codon after 17 amino acids in ORF-8 only from these 93,265 genome sequences were analysed (Workflow: Step C).

3. Results

3.1. Workflow of mutation analysis

We have used the following workflow for the mutation analysis (see Fig. 1).

3.2. Common SARS-CoV-2 mutations across the globe

We analysed total 15,120 full-length SARS-CoV-2 genome sequences (global set), available on the NCBI database as on 25 June 2020, investigating mutations occurrence since COVID-19 outbreak. The alignment was made with the viral reference strain (accession number NC_045512.2) of the virus. We detected 11 synonymous and non-synonymous mutations from the global set of data (Table 1 and Supplementary Fig. 1), these mutations were previously reported (Phan, 2020; Jul; Tang et al., 2020; Wu et al., 2020; Stefanelli et al., 2020; Pachetti et al., 2020 Dec 22). From these 11 mutations the C241T mutation is occurring in 5’-UTR regions of the virus genome. The mutation was identified in quite significant number of samples (over 85% in all countries except China USA and Spain where it was 3%, 40% and 56%, respectively). Three of the 11 mutations are synonymous mutations. The remaining 8 lead to protein change in open reading frames 1a, 1b, S, 3a and 8 (Table 1, Supplementary Fig. 1).

3.3. Unique mutation in the SARS-CoV-2 samples from each continent

We were also interested in mutations occurrence in a minute percentage of samples globally. We have chosen Australia as representative of Oceania continent, China, India and KSA as representative of Asia, Egypt representing Africa, Italy and Spain as representative of Europe, USA for North America, Mexico for Central America and Brazil as a representative of South American continent. The chosen countries were having quite notable numbers of SARS-CoV-2 infection cases. We used the GISAID database to download the 100 full-length genome sequences derived from the above-mentioned countries, selected at random. Alignment of these samples to reference strain showed us a total of 313 mutations in SARS-CoV-2 genome in country set of data (Supplementary Table 1). Many of these mutations are rare but a considerable number of mutations are common in samples derived from all six continents (Table 2). All the 11 mutations found in the global set of data were also included in the 313 mutations, obtained from the country set of data.

3.4. Country specific mutations

The 313 mutations were found at the nucleotide level in most of the ORFs, except in ORF 7b, 10 and 3’-UTR (Fig. 2). The highest mutation occurrence (78 mutations; 24.9%) were detected in Australian samples, whilst the lowest mutation occurrence compared to reference strain, were found in Brazilian samples (28 mutations; 8.9%) (Fig. 2 and Supplementary Table 1).

3.5. Mutations at amino acid level

All 313 mutations were analysed, for their corresponding amino acid changes, as shown in (Fig. 3). The highest non-synonymous mutations (43 mutations; 13.7%), we detected in Australian samples, which corresponds to the highest nucleotide mutations (78 mutations). While the lowest non-synonymous mutations were found in KSA derived samples (17 mutations; 5.4%).
3.6. Most of the nucleotide mutations are non-synonymous mutations

We noticed that non-synonymous mutations are higher than synonymous mutations in majority of ORFs (Fig. 4). We discovered 2% of the genome sequences in Australian samples have insertion of TTT at position 11,085 which lies in ORF-1a. The mutation adds one amino acid, F at the 3607 position in the ORF-1a, more precisely, the mutation adds F amino acid at the position 38 in non-structural protein 6 (NSP6), which

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Fig. 1. Workflow of identification of mutations in SARS-CoV-2 genome sequences.
expresses as part of ORF-1a. Another independent mutation that interests us at the position C27945T in 2% Italian samples, which translate to non-sense codon in the ORF-8. The mutation creates premature stop codon and does not allow the ORF-8 to express after the 17 amino acid although it is 121 amino acid long ORF, as per the reference strain (Supplementary Fig. 3).

3.7. The occurrence of nsp6 insertion mutation predominantly in UK derived samples

We analyzed the occurrence of 11085TTT insertion mutation in the nsp6 in the GISAID. From the analysis of 93,265 SARS-CoV-2 genome sequences, we found the insertion mutation in the nsp6, only in 288 samples. Remarkably, out of the 288 samples, 190 (65.97%) samples were derived from UK patients. Australia derived samples are the second-largest (8.3%) after the UK, with the insertion mutation (Fig. 5, Table 1). Common SARS-CoV-2 Mutations Across the Globe.

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Nucleotide</th>
<th>Occurrence</th>
<th>ORF</th>
<th>Amino Acid</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>C241T</td>
<td>61%</td>
<td>5′-UTR</td>
<td>N/A</td>
</tr>
<tr>
<td>2</td>
<td>C1059T</td>
<td>28%</td>
<td>a</td>
<td>T265I</td>
</tr>
<tr>
<td>3</td>
<td>C3037T</td>
<td>52%</td>
<td>a</td>
<td>Silent</td>
</tr>
<tr>
<td>4</td>
<td>G2782T</td>
<td>31%</td>
<td>a</td>
<td>Silent</td>
</tr>
<tr>
<td>5</td>
<td>C14480T</td>
<td>52%</td>
<td>b</td>
<td>P314L</td>
</tr>
<tr>
<td>6</td>
<td>G17747T</td>
<td>26%</td>
<td>b</td>
<td>Y1464C</td>
</tr>
<tr>
<td>7</td>
<td>A17858G</td>
<td>26%</td>
<td>b</td>
<td>Silent</td>
</tr>
<tr>
<td>8</td>
<td>C18060T</td>
<td>26%</td>
<td>b</td>
<td>Silent</td>
</tr>
<tr>
<td>9</td>
<td>A23403G</td>
<td>37%</td>
<td>S</td>
<td>D614G</td>
</tr>
<tr>
<td>10</td>
<td>G25563T</td>
<td>38%</td>
<td>3a</td>
<td>Q57H</td>
</tr>
<tr>
<td>11</td>
<td>T26114C</td>
<td>25%</td>
<td>8</td>
<td>L845</td>
</tr>
</tbody>
</table>

Table 2
The prevalence of mutations in samples derived from representative countries of all six continents.

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Mutation</th>
<th>Australia</th>
<th>China</th>
<th>India</th>
<th>KSA</th>
<th>Egypt</th>
<th>Italy</th>
<th>Spain</th>
<th>USA</th>
<th>Mexico</th>
<th>Brazil</th>
<th>ORF</th>
<th>Amino Acid</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>C241T</td>
<td>85%</td>
<td>3%</td>
<td>94%</td>
<td>90%</td>
<td>100%</td>
<td>56%</td>
<td>40%</td>
<td>86%</td>
<td>100%</td>
<td>5′-UTR</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>C1059T</td>
<td>13%</td>
<td>2%</td>
<td>9%</td>
<td>9%</td>
<td>100%</td>
<td>4%</td>
<td>28%</td>
<td>12%</td>
<td>15%</td>
<td>1a</td>
<td>T265I</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>C3037T</td>
<td>90%</td>
<td>3%</td>
<td>98%</td>
<td>90%</td>
<td>100%</td>
<td>57%</td>
<td>42%</td>
<td>86%</td>
<td>100%</td>
<td>1a</td>
<td>Silent</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>C3782T</td>
<td>5%</td>
<td>48%</td>
<td>8%</td>
<td>2%</td>
<td>15%</td>
<td>5%</td>
<td>4%</td>
<td>15%</td>
<td>1a</td>
<td>L3606F</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>G11083T</td>
<td>9%</td>
<td>6%</td>
<td>90%</td>
<td>65%</td>
<td>100%</td>
<td>56%</td>
<td>40%</td>
<td>86%</td>
<td>100%</td>
<td>1b</td>
<td>P314L</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>C14408T</td>
<td>88%</td>
<td>3%</td>
<td>90%</td>
<td>92%</td>
<td>100%</td>
<td>56%</td>
<td>40%</td>
<td>86%</td>
<td>100%</td>
<td>1b</td>
<td>P314L</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>C18050T</td>
<td>6%</td>
<td>31%</td>
<td>2%</td>
<td>15%</td>
<td>1b</td>
<td>35%</td>
<td>7%</td>
<td>1b</td>
<td>1b</td>
<td></td>
<td>Silent</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>C17747T</td>
<td>5%</td>
<td>35%</td>
<td>7%</td>
<td>1b</td>
<td>1b</td>
<td>35%</td>
<td>9%</td>
<td>1b</td>
<td>1b</td>
<td></td>
<td>Silent</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>A23403G</td>
<td>80%</td>
<td>5%</td>
<td>90%</td>
<td>98%</td>
<td>100%</td>
<td>60%</td>
<td>40%</td>
<td>86%</td>
<td>100%</td>
<td>1a</td>
<td>D614G</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>G25563T</td>
<td>20%</td>
<td>4%</td>
<td>50%</td>
<td>70%</td>
<td>4%</td>
<td>35%</td>
<td>14%</td>
<td>3a</td>
<td>1a</td>
<td>G2571</td>
<td></td>
<td></td>
</tr>
<tr>
<td>11</td>
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<td>15%</td>
<td>5%</td>
<td>2%</td>
<td>15%</td>
<td>3a</td>
<td>15%</td>
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<td>1b</td>
<td>G2571</td>
<td></td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>C26735T</td>
<td>5%</td>
<td>50%</td>
<td>60%</td>
<td>3%</td>
<td>1b</td>
<td>1%</td>
<td>2%</td>
<td>1b</td>
<td>1b</td>
<td>S</td>
<td>Silent</td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>G28881AA</td>
<td>60%</td>
<td>1%</td>
<td>1%</td>
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<td>40%</td>
<td>6%</td>
<td>5%</td>
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<td>N</td>
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<td>14</td>
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<td>1%</td>
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<td>23%</td>
<td>21%</td>
<td>40%</td>
<td>6%</td>
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<td>G204R</td>
<td></td>
</tr>
</tbody>
</table>

Fig. 2. SARS-CoV-2 Mutations in different ORFs The graph shows the total mutations in the 5′-UTR and various ORFs of the SARS-CoV-2 genome.
3.8. The occurrence of non-sense mutation in the ORF-8 in Europe and USA derived samples

We also investigated the occurrence of the C27945T mutation that translate to non-sense codon after 17 amino acid in ORF-8. The search resulted to 67 samples, out of 93,265 SARS-CoV-2 genome sequences obtained from the GISAID database, with the C27945T mutation. 97% of these samples were derived from Europe and USA, (Fig. 6 A, 6B and Supplementary Table 3). Only two samples were derived from non-western countries; one from Singapore and the another from South Africa.

4. Discussion

The RNA genome of SARS-CoV–2 renders the virus prone to mutations. We report novel mutations in the SARS–CoV–2 genome resulting in change in 5′-UTR or translational change, and subsequently altered proteins. The virus appears to be mutating across the globe and some of these mutations are arising independently across various populations and locations.

Many of the mutations found are common in samples, derived from more than one country or continent, as shown in Table 2 and Supplementary Table 1. The result in Table 1 indicates the mutations are highly skewed towards C-to-T transitions (>70%), this mutation bias might be caused by host-specific cytidine deaminases, mostly APOBEC3. The
cytidine deaminases systems are directly involved in virus mutagenesis and this study confirms this general trend observed in early studies (Matyášek and Kovářík, 2020; Simmonds, 2020 Jun 24).

Three mutations (C186T, A187G and C241T) were found in 5′–UTR. The C241T mutation is very common and previously reported (Stefanelli et al., 2020), the C186T mutation occur only in 3% of samples derived from main land China and the A187G mutation found in 2% of samples derived from Italy. These two independent mutations were not observed commonly in any other population, suggesting a localised non–advantageous change. The C241T mutation in 5′–UTR, was observed in 7 countries out of 10 with a frequency of more than 85%. The exceptions being China, USA and Spain where the frequency was as low as 3%, 40% and 56%, respectively. The mutation may be providing replication advantage to the virus and the mutant virus is prevailing over time. A detailed study of the C241T mutation is required to shed light on its role in infectivity or pathogenesis. Most of the identified mutations occur in ORF1ab, which is concordant with expectations of a long ORF, however, ORF–8 and ORF–N, despite their relatively small size, have a higher density of mutations as seen in Fig. 4 and Supplementary table 1.

The two mutations (C17747T and A17858G) previously reported only in the USA derived samples (Pachetti et al., 2020 Dec 22), were also found in the current study with a frequency of 35% in USA samples and 7% in Mexican samples. This may be reflective of the geographical proximity of the countries and thus more frequent movement of the population. The other five mutations (C1059T, C3037T, C14408T, C18060T and A23403G), were previously reported as restricted to European derived samples (Pachetti et al., 2020 Dec 22), however, our findings indicate that these mutations are spreading in all six continents in quite significant numbers of samples over time (Table 2 and Supplementary table 1).

The A23403G mutation is translating into D614G in ORF–S, which has been observed globally (Korber et al., 2020) and correlated with mortality rate in COVID–19 patients (Toyoshima et al., 2020 Dec 22). The frequency of the D614G mutation in ORF–S, was found to be more than 80% in 7 out of 10 countries, except China, USA, and Spain, where it is 3%, 40% and 60%, respectively. The frequent simultaneous occurrence of two mutations at the opposite ends of the genome two (C241T and A23403G) is notable. However, in the literature, there is no information on the correlation of these mutation with the mortality rate and human pathology.

Out of the 313 mutations observed, the majority (169 mutations) are non-synonymous mutations. These non-synonymous mutations were found in all ORFs (except ORF 7b, 10 and 3′–UTR) and thus highly likely to affect the infection and pathogenesis of the virus profoundly.

Fig. 5. The occurrence of insertion mutation in SARS-CoV-2 nsp6 The graph shows the occurrence of 1108STTT insertion mutation in SARS-CoV-2 genomes globally.

Fig. 6. The occurrence of non-sense mutation in SARS-CoV-2 ORF-8 The graphs show the occurrence of C27945T (non-sense) mutation in ORF-8.
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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.gene.2022.147020.

References


