Analysis of SARS-CoV-2 spike protein mutations in the UK

Abstract

Introduction

**Background: COVID-19 pandemic**

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is the strain of coronavirus responsible for the ongoing COVID-19 pandemic (Hu et al., 2021). SARS-CoV-2 was initially detected in Wuhan, China, and has now spread to all corners of globe (Huang et al., 2020). Approximately 770,000,000 people have contracted the virus and over 7,000,000 people have died due to complications caused by the virus (Anon). This figure is most likely even higher as a result of governments’ inability to report cases, as well as, corruption with countries attempting to hide the true values. An estimated value of the number of deaths sits at around 18,000,000 – 32,000,000 (Anon). The economic burden has been far-reaching, estimated losses are up to $16 trillion worldwide and unemployment levels have soared (Cutler and Summers, 2020). Nationwide lockdowns, that existed at the height of the pandemic, have not only had an economic effect, but, have also impacted education and literacy rates. It has been predicted that by 2030 less than 40% of pupils in the UK will achieve a pass in GCSE English and Mathematics (Major et al.). SARS-CoV-2’s effects will continue to resonate, even as the virus becomes less prevalent.

SARS-CoV-2 is the most recent coronavirus (CoVs) that has impacted humanity. Human coronaviruses (CoVs), HCoV-229E and HCoV-OC43, have co-existed for centuries (Pyrc et al., 2006). These viruses result in far more mild symptoms similar to that of the common cold. This is in stark comparison with severe acute respiratory syndrome coronavirus 1 (SARS-CoV-1), Middle East respiratory syndrome – related coronavirus (MERS-CoV) and severe acute respiratory coronavirus 2 (SARS-CoV-2), which are all highly pathogenic. This high pathogenicity exists due to the viruses targeting important cellular tissues, bronchial epithelial cells and tissue in the upper respiratory tract (Marik et al., 2021). This where the angiotensin-converting enzyme 2 is expressed (Li et al., 2020). SARS-CoV-1, MERS-CoV and SARS-CoV-2 have emerged over the past 25 years, with several outbreaks occurring. Most notably the SARS-CoV-1 2002-2004 outbreak, 2015 MERS outbreak in South Korea and the COVID-19 pandemic as a result SARS-CoV-2 (Arora et al., 2020). There appears to be a growing trend in the in the prevalence of coronaviruses outbreaks, even though the rates of SARS-CoV-2 have greatly diminished (Anon). This stresses the importance of studying SARS-CoV-2 and other related to viruses, so that globally we are better prepared to combat the next epidemic or pandemic.

**Background: SARS-CoV-2 structural and molecular information**

Coronaviruses are of the order Nidovirales, which is comprised of several families of related viruses (Fehr and Perlman, 2015). CoVs are enveloped positive-sense single-stranded RNA viruses (Yang and Rao, 2021). Known for having a high mutation and recombination rate, CoVs unlike most RNA viruses have a genetic exonuclease proofreading mechanism (Cui, Li and Shi, 2019). This genetic proofreading mechanism would usually lead to a high fidelity rate and potentially lower mutation rate, however, a high mutation rate still exists. SARS-CoV-2 enters the human cell by binding to several different cellular entry receptors, such as, angiotensin-converting enzyme 2, through its spike protein (Hoffmann et al., 2020).

**Background: Spike glycoprotein**

The spike glycoprotein is one the main structural components SARS-CoV-2. A homotrimer composed of two regions, the S1 and S2 regions, vital for binding and cellular fusion (McCallum et al., 2020). The ACE2 – spike protein interaction plays a vital role in the infectivity of SARS-CoV-2. The interaction is vital as it has been shown that the binding free energy change between the host ACE2 and the spike protein is proportional to the infectivity of SARS-CoV-2 (Wang et al., 2021). The spike protein receptor binding domain (RBD) of the S1 subunit catalyses the attachment directly to the ACE2. More specifically, residues of the receptor binding motif are involved directly in the binding (Chen et al., 2020). This emphasises the biological importance of the spike protein as mutations to residues on the protein can potentially increase or decrease the infectivity of novel strains of SARS-CoV-2.

**Aims of the investigation:**

Analysis was completed on 1984861 individual UK SARS-CoV-2 spike protein sequences stored in GISAID. This study aimed to assess the general dataset trends was performed to provide information on the sequencing activity, number of mutations and the emergence of novel mutations. Clustering and distribution analysis were performed to classify SARS-CoV-2 spike protein mutations based on sequence and track the geographical distribution of the virus strains. Further analysis focused on characterising the spike protein mutations. With a particular interest in distinguishing between synonymous and non-synonymous, to assess their implications. The potential effects of the most common mutations on viral infectivity have also been considered. Outlining their location within the receptor binding domain (RBD) and receptor binding motif (RBM), to understand the impact on protein functionality following mutations at these vital residues. The study looks to provide an insight into the trends of SARS-CoV-2 spike glycoprotein mutations in the UK and to elucidate changes in viral infectivity.

1. **Provide a complete overview of the general dataset trends**
2. **Investigate the clustering and distribution of SARS-CoV-2 spike protein mutations in the UK**
3. **Identify and characterise common spike mutations + non vs syn mutations**
4. **Analyse their potential effects on viral infectivity, link to receptor binding domain/receptor binding motif**

**Methods overview**

Dataset used was provided by Dr Richard J Bingham, at the University of York, which was obtained from GISAID. Clustering techniques used were k-means and t-distributed stochastic neighbour embedding (t-SNE), to assess the distribution of viral variants. Many R packages were used, including the tidyverse and ggplot, which provided the basis for the majority of the R analysis. A link to a GitHub repository containing the research compendium can be found in the methods section.

1. **Summarise the dataset used**
2. **Mention of the clustering techniques used, R-based analysis, various packages used and tools used for visualisation**

**Summary of the main findings**

**The number of mutations per sequence increases over time**

Results

**Introduction to the dataset**

The analysis is based on the complete SARS-CoV-2 genome sequences deposited in GISAID, as previously mentioned. The dataset includes sequence information such as a unique identifier, sample date, and country of origin, along with specific mutations for each sequence. The dataset was manipulated to provide an overview and identify general trends in the data. The resulting 1984861 genome sequences from across the UK, provided 15213 unique single mutations.

**Sequencing efforts**

Figure 1A-F illustrates the number of sequences per day from 2020 – 2024. 78% of sequences originated from England, 14% from Scotland and 8% from Wales. Figure 1A provides an overview of the sequencing activity, highlighting peaks associated with the emergence of new SARS-CoV-2 variants and a decline in sequencing as COVID-19 cases decreased. Sequencing activity peaked at the end of 2021, Fig 1C, and the beginning of 2022, Fig 1D, reflecting the rapid spread of new variants and the need to characterise them. It is interesting to note the reduced sequencing efforts after mid-2022 to 2024 as SARS-CoV-2 becomes less prevalent. This reduction may be in relation to reduced testing, herd immunity or changes in public policies. A baseline of testing continues across the UK. Fig 1B, gradual increase in sequencing activity. Fig 1C: rapid increase in the number of sequences per day, coincides with the emergence of new variants, **such x and y**. Fig 1D sharp increase in sequencing activity, peaking at the start of the year, this peak is likely due to the emergence of the omicron variant, followed by a sharp decline in the number of sequences per day. Fig 1E-F, overall sequencing activity is very low, indicating reduced viral spread. By highlighting the sequencing trends, this figure provides a timeline of the spike protein mutations and insights into the emergence of novel SARS-CoV-2 variants in the UK.

A screenshot of a graph

AI-generated content may be incorrect.

Want to change this plot, to have curved lines showing the emergence of new strains

Use a model to show to the relationship between the number of sequences per day and the sample date

A colorful chart of a graph

AI-generated content may be incorrect.

**Overview of the mutations**

Following this overview of the sequencing activity, the general trends linked around the mutations were also investigated. Figure 2 shows the distribution of mutations per sequence over time (2020 – 2024) using a violin plot. A violin plot helps to illustrate the overall shape of the data and identify the variation that exists within this dataset. The shape of the violin can be interpreted in that the wider sections indicate more observations and the narrow sections represent fewer observations. The median number of mutations per sequence increases from 2020 – 2024, implies that as time progresses the sequences accumulate more mutations. In 2021, the maximum number of mutations for a sequence was 166. In 2022, a sequence had 136 mutations, yet, the maximum values for the other years was close to half of these values. This trend is possibly linked to the sequencing efforts during those years. Fig 1C and Fig 1D depict increased sequencing activity; this increased activity will lead to a higher probability of obtaining extreme values, due to the larger number of samples being taken. A Kruskal-Wallis test followed by post-hoc analysis showed that there were significant differences between the years in terms of number of mutations per sequence; this implies that sequences are progressively accumulating mutations over time. A Spearman’s rank correlation value of 0.895 further supports the notion that as time progresses mutations per sequence increase.

A graph of different colored squares

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15213 unique individual mutations were detected in the dataset.

Figure 3, Number of unique mutations over time

Number of novel mutations per day

**Clustering and the distribution of mutations**

1. **Geographic distribution of spike protein mutation**

Figure, map of the distributions

1. **Map visualising the distribution in the UK**

**Mutation analysis**

What I did?

1. **Summary of the most common mutations**

**Figure x**. The top 20 most common spike protein mutations. **A** Plot of the most common spike protein mutations. Blue colour indicates nonsynonymous and red colour indicates synonymous mutations. **B** The 3D structure of the SARS-CoV-2 spike protein with the top 20 most common mutations marked on their respective residues.

Top 20 most common mutations:

non\_A23403G~D-G, non\_C22995A~T-K, non\_G21987A~G-D, non\_A23063T~N-Y, non\_C23604A~P-H, non\_C21846T~T-I, non\_T22917G~L-R, non\_G22992A~S-N, non\_C23525T~H-Y, syn\_C25000T, non\_G23948T~D-Y, non\_T22679C~S-P, non\_T24469A~N-K, non\_T23599G~N-K, non\_A24424T~Q-H, non\_C23854A~N-K, non\_C22686T~S-F, non\_A23055G~Q-R, non\_T23075C~Y-H, non\_T22882G~N-K

How many of these mutations are on the receptor binding domain?

How many of these mutations are on the receptor binding motif?

Why is there a syn mutation?

What is a synonymous mutation and what is nonsynonymous mutation?

1. **Non vs syn mutations**
2. **Frequency of mutations of most common mutations**

**Evolutionary Insights**

1. **Phylogenetic tree highlighting relationships among clusters**

**Impact of mutations**

1. **Link mutations to known functional effects**

Discussion

**Interpret the results**

1. **Correlation between geographical distribution and specific mutations**
2. **Mutation hotspots? Natural selection + selective pressure**

**Link back to previous studies**

**Implications for public health**

1. **Vaccine design**
2. **Impact of mutations on diagnostics and therapeutic intervention**

**Limitations and future directions**

1. **Limitations of the dataset**
2. **Limitations of techniques used**
3. **Future research**

Conclusion

**Summary of the main findings**

**Importance of studying SARS-CoV-2**

Methods and Materials

**Data sources**

**R packages used**

**Statistical analysis**

**Any additional stuff used**

**Link to GitHub:** [**sha524/Spike\_protein**](https://github.com/sha524/Spike_protein)

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