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12/14/2021

Genetic mutation and variants of SARS-CoV-2 and their relative effect on transmission, morbidity, and mortality: a systematic review

Introduction

A new strain of coronavirus, SARS-CoV-2 emerged in December of 2019. Due to its high transmission rate, it was able to spread rapidly across the world, resulting in a global pandemic. As scientists grappled with understanding, treating, and vaccinating against the disease, continual surveillance was done to track new variants that could evade treatment vaccine or had the potential to be more lethal (Lokman et al., 2020). The virus SARS-CoV-2 stands for Severe Acute Respiratory Syndrome Coronavirus 2, and based on phylogenetic studies, it belongs to the genus beta-coronavirus, which also includes the SARS coronavirus that once caused a smaller pandemic in China in 2003 (Gorbalenya et al., 2020). SARS pandemic spread throughout mainland China for 7 months, infecting 5318 persons in 194 administrative regions (Cao et al., 2016). The common symptoms of SARS-CoV-2 observed in clinical practice include major ones such as fever, cough, and dyspnea, minor ones such as headache and gastrointestinal symptoms, and notably the loss of taste and smell in many patients. (De Vito et al., 2020, 2021).

Since the pandemic, data scientists, biologists, and evolutionary biologists across the globe have collaborated and strove very hard to provide a holistic image of proteomic and genomic variation data of SARS-CoV-2 (Jary et al., Alvarez-Diaz et al., Lokman et al., 2020). A lot of mutants and key strains potentially were the focus of interest. For example, one of the most prevalent variants during the early development stage of SARS-CoV-2 was the delta 382 variant, which indicates a 382-nucleotide deletion in the ORF8 gene. Besides the mutation, a key mechanism regarding the pathway in which SARS-CoV-2 invades human cells was identified: mutations on the surface of the S spike protein also play a crucial role for its responsibility involved in virus-host through ACE 2 interaction (Su et al., Gong et al. 2020). This review aims to provide relevant research which investigates SARS-CoV-2 variants. The research will, either individually or collectively, characterize features among different variants, highlight the effects of different variants on transmission, and also discuss their effects on clinical morbidity, and mortality. Along with the discussion, the underlying mechanisms, if known, will also be described. This review will not include analysis into any strain specifically.

Methodology

This study is a systematic review of current evidence related to the topic until June 2021. 17 primary research papers and 3 medical studies, all published in English, were retrieved using systematic search by keywords in the online database including PubMed, bioRXiv, and medRXiv. Research from all relevant papers included in this study used data from all over the world, including China (the origin outbreak place of SARS-CoV-2) to regions including the Middle East, South America, and Europe, etc. The search strategy employed searching with different combinations of keywords, described as follows:

- "SARS-CoV-2", "Coronavirus", "COVID-19", "new Coronavirus", "novel Coronavirus"
- "Mutants", "Variants", "Genetic variations", "Genomic variants", "Strains", "Allele variants"
- "Phylogenetic", "Genomic sequencing", "Proteomic sequencing", "Evolution"

Selection of studies

SARS-CoV-2 is identified as a virus with a relatively low mutation rate (Manzanares, Medina, 2020). Despite the nature of SARS-CoV-2, the papers selected for this study are still aimed at giving a full description of mutations updated to the latest information, which includes the studies focused on various geographic locations. Based on the search method above, the full texts of the studies were extracted and filtered through the following exclusion criteria:

- Non-human studies
- Studies without referencing this report's keywords
- Papers with the inaccessibility of the full texts

It should be also noted that publication date is an important factor when selecting studies for. This review reveals the evolution pathway of SARS-CoV-2, and the more recent a study is the more urgent the needs of global surveillance and public attention are. Besides, although peer-review is an important process in deciding the credibility, reliability, and reproducibility of a research article, the selection of research paper in this review will not specifically select for the peer-review articles but also include the preprints.

Extraction of data

Information including the authors' name, publication date, focused variants, study method, and main results were collected and will be organized into an independent table. Data nature is also a key point to notice when filtering valuable information: for example, data derived

using an epidemiology approach should be handled and analyzed differently when compared to experimental data.

Source of data

Most of the studies included deriving SARS-CoV-2 genome sequence data from a free, public database website, GISAID (GISAID - Initiative). Whether the data source is public or private one can be important in the study process with potential differences in genome data quality, data accuracy, and completeness of the consent process.

Credit is also given to NextStrain (ncov / open / global) for its extremely extensive data on tracing genomic variation of SARS-CoV-2 with high accuracy on data interpretation, as well as drawing the phylogenetic relationship tree among variants. Enabling its data from GenBank, the NIH genetic sequence database (GenBank Overview), NextStrain collects 3277 genome samples between Dec 2019 to Dec 2021. For each lineage, the database identifies nucleotide mutation, amino acid mutation, and the extent of divergence from the original SARS-CoV-2 strain.

Discussion of Findings

Since the emergence of SARS-CoV-2, many relevant aspects of SARS-CoV-2 such as virus behavior, infection rate, transmission rate, mutation rate, and potential future treatment have gained attention from all over the world. Continuously emerging mutations contribute to different virus behaviors and therefore lead to greater threats to human health.

This section will be the discussion of different SARS-CoV-2 variants based on the supplementary table which summarized the main findings of research articles included in this review. The result table features the investigated mutant or mutation and researchers' important findings relating the variant to the broader human public health. This part will be divided into three sections: characterization of different SARS-CoV-2 variants, their effects on virus-human transmissibility, and their effects on clinical morbidity and mortality.

Different variants and their major characteristics

The review focuses on characterizing variation in the SARS-CoV-2 genome and also highlighting the effects of different types of mutation on infectivity, disease severity, and viral life cycle. Jary et al (2020) used next-generation sequencing to identify the dynamic distribution of SARS-CoV-2 variants and demonstrate that SARS-CoV-2 has a low mutation rate. Tracing the

dynamic distribution of variants looks at the real-time spread of variants of concern, making it a helpful tool also in the field of epidemiology, given its ability to meanwhile summarize the change in the spread of mutants. During the early development stage of SARS-CoV-2, there were two major types of genotypes identified in China: type I and type II. The researchers analyzed 169 genome samples from online databases and applied genome alignment to determine the degree of similarities across the samples. While type II was the more predominant strain in Chinese patients, the strain was found to have evolved from the ancestral type I strain based on phylogenetic analysis. Later research into type I and type II strains found that among three distinguishable variation sites between type I and type II, two sites have synonymous variations that lead to higher amino acid translation frequency, suggesting the possibility of type II strain can produce virus particles faster and spread more rapidly in the host cells (Zhang et al., 2020).

Further studies focusing on amino acid change have also identified three major mutant types of SARS-CoV-2 (Al-Tawfiq et al., 2020). Forster et al. confirmed the three mutant types (A, B, C) by phylogenetic analysis of 160 viral genomes, sampled from clinical data in Germany. It is found that type A is the ancestral type, and type B evolved from type A by two amino acid mutations: the synonymous mutation T8782C and non-synonymous mutation C28144T, which changes a leucine to a serine. In addition, type C has also evolved from the parent type B by a non-synonymous mutation G26144T, changing a glycine to a valine. The non-synonymous mutations are defined by the changes in the DNA base which do not affect the amino acid sequence, and this nature often accompanies more alerts and attention from scientists. From epidemiology data in this study, type B appears to be prevalent in Asia, whereas type A and type C were found in significant proportions in North America and Europe (Forster et al., 2020). The study suggests that the two mutations that differentiate type B from its ancestral type A strain may explain why type B is only common in Asia. However, the study is heavily and solely based on phylogenetic analysis, and more evidence is needed to testify the association between these two mutations and the prevalence of type B among Asian countries.

Besides the general description of mutated variants, two noteworthy mutations lead to significant changes in mutants in the global scope: C14408T mutation on Nsp12 and A23403G mutation on S spike protein. Both are missense mutations: C14408 T causes P323L exchange causing a missense mutation; A23403G, which the researchers mentioned being “one of the most important mutations”, results in D614G substitution on S spike protein. It is also noted that a previous study has also mentioned the crucial role of S spike protein in viral entry into the host cell (Gordon et al., 2020; Ou et al., 2020). It is also found that these two mutations are responsible for the structural conformation change required for receptor binding. In particular, Bhowmik et al also demonstrated that the mutation on S spike protein has a strong association with human-human transmission rate through the interaction between SARS-CoV-2 and hosts’ ACE 2 receptor (Bhowmik et al., 2020). The researchers collected more than 30,000 genome samples from different continents and despite some variations in low-frequency rate in some

continents, these two mutations were observed, often simultaneously, across the world, being the most prominent mutations in the SARS-CoV-2 genome (Ugurel et al., 2020).

Recent studies have also identified eight major SARS-CoV-2 mutated strains, but they largely share commonalities. The mutation spots are located at positions 1397, 2891, 14408, 17746, 17857, 18060, 23403, and 28881. Among them, mutations in 2891, 3036, 14408, 23403, and 28881 are prevalently observed in European patients, whereas those located at positions 17746, 17857, and 18060 are exclusively present in North America areas. The team concludes that the coronavirus is evolving and that strains from Europe, North America, and Asia can co-exist together whereas each strain retains its distinctive mutation pattern (Pachetti et al., 2020).

While efforts in filtering concerning variants that pose potentially great threat worth credits, some researchers also approach investigation by comparing SARS-CoV-2 to its ancestry which once caused localized endemic in China, SARS-CoV-1. For example, Korber's team reviewed the finding of an identified amino acid mutation of SARS-CoV-1 in a research paper published in 2008. The finding regards the amino acid mutation on the S spike protein of SARS-CoV-1, which was Spike D480A/G in the receptor-binding domain (RBD). This mutation led to the new strain becoming the dominant variant among 2003/ 2004 viruses (Sui et al., 2008). Korber's team, therefore, proposed that SARS-CoV-2 may also acquire similar mutations with fitness advantages and immunological resistance, leading to their discovery of greater infectivity of the G614 variant (2020).

The effects of variants on transmissibility

Fortunately, under global collaboration, scientists and researchers have identified several mutations involved in the facilitation process of human-human transmission. After analyzing 1271 SARS-CoV-2 genome sequences from the public database NCBI NR, Panchin et al. reported that when comparing the SARS-CoV-2 sequence to the coronavirus sequence obtained from bats, 9-fold excess of G-U transversions has been found. This result indicates that the transmission pattern has changed after the virus has mutated to infect humans. In addition, the pattern became less obvious when comparing sequences of SARS-CoV-2 and SARS-CoV, suggesting that G-U transversion is associated with the transmissibility of the whole SARS-CoV family. However, the team discovered that this transversion pattern is not symmetric: there is no sign of C-A mutations in the complementary strand (when the virus genome gets duplicated) (Panchin et al., 2020).

It is also suggested that the viruses that cause mild symptoms in patients, compared to those strains of viruses that lead to a greater morbidity rate in clinical practice have a longer transmission process. The researchers mentioned that the evolution of SARS-CoV-2 remains

poorly understood, and the team focused on the evolution theory behind it and posited that the rapid transmission process will favor more pathogenic strains and vice versa. This is because of the living nature of viruses that viruses cannot maintain life features outside the hosts; residence inside hosts is beneficial for virus regeneration. Therefore, in evolution theory, the slow-replicating strains which cause only mild symptoms but not deadly or acute conditions will allow for a longer transmission process, persisting longer in the population (Blackstone et al., 2020)

More importantly, the researchers are also able to identify the gene and a specific mutation that are responsible for higher and faster transmission. According to Korber's team, following Ugurel's finding of D614G mutation and its prevalence in the world, they track variant frequencies of the pattern of G614 increase on multiple geographic levels such as national, municipal, and regional. It is found that the rise of local epidemics is associated with the introduction of D614G mutation. Data presented also identified three other mutations accompanied with D614G: The D614G change is almost always accompanied by three other mutations: a C-to-T mutation in the 5′ UTR (position 241 relative to the Wuhan reference sequence), a silent C-to-T mutation at position 3,037, and a C-to-T mutation at position 14,408 that results in an amino acid change in RNA-dependent RNA polymerase (RdRp P323L). The altogether four haplotypic changes were linked to the finding that the G614 variant was the globally dominant form, suggesting that the G614 variant has a fitness advantage and has led itself to become more powerful in infected individuals.

Epidemiological research was conducted by probing the prevalence of the D614 variant and G614 variant across the globe. It was found that before March 1, 2020, G614 was found in 10% of 997 global sequences; between March 1 and March 31, 2020, it represented 67% of 14,951 sequences; and between April 1 and May 18, 2020 (the last data point available in the May 29, 2020 sample), it represented 78% of 12,194 sequences. Lab results from RT-PCR also demonstrated the greater magnitude of infection appeared by G614 mutant, where researchers found that samples taken from individuals infected by G614 variants reached a lower CT threshold, indicating larger viral loads. The lab results also supported the hypothesis that the G614 variant is associated with higher infectious titers of spike-pseudotyped viruses, however, is not related to disease severity (Korber et al., 2020).

Fortunately, the underlying mechanism for which the process of D614G mutation leads to greater infectivity is also identified. There are other pieces of supporting evidence demonstrating the relationship. Zhang et al discovered that G614 increases the stability of S spike protein and membrane incorporation, further emphasizing the function and possible outcomes of G614 mutation on the transmission process. The viral-host interaction mechanism is mediated by the initial binding of the receptor-binding domain presented at the S1 unit of S spike protein to the hosts’ ACE 2 receptor (Bhowmik et al., 2020). Although S spike protein from the G614 variant

does not bind to ACE2 more efficiently than S spike protein from D614, Zhang mentioned that the G614 mutation achieves its greater infection activity by stabilizing the interaction between S1 and S2 domain units and reducing S1 shedding. Once S1 shedding is limited, the virus can create more stable binding sites and allow for more efficient transmission between SARS-CoV-2 and humans (Zhang et al., 2020).

Another important variant is the Delta (B.1.617.2) variant. According to NextStrain, there are 8 mutations on the Si unit of the spike protein in the Delta variant. Several nucleotide and amino acid mutations were identified, including C7124T, A11201G, A11332G, and P2287S, T3646A mutations on ORF1a. Sequencing data and study of prevalence were carried out to demonstrate that the Delta variant has an estimate of increased transmissibility (Dagpunar, 2021).

Infectivity and pathogenicity of SARS-CoV-2 are highly related to S spike protein. The interaction between virus and ACE2 receptor is due to binding ridge structural changes of the receptor-binding domain on residues 482-485: Gly, Val, Glu, Gly (Wu et al., Shang et al., 2020). Poterico et al identified four changes in the genetic region responsible for primer annealing for SARS-CoV-2 specific fragment identification: nt15324 in ORF1ab (RdRp), nt26144 in E gene, and nt28580 and nt28657 in the nucleocapsid gene (2020). Besides key specific amino acid mutation, it is worth noticing that two nucleotide changes in genes ORF1ab (nt8750) and N (nt29063) are also responsible for higher transmission rates of SARS-CoV-2 by having higher translational efficiencies (Zhang et al., 2020). Poterico's team included that genes ORF1ab and N genes are associated with the fact that clade G strain is a lot more contagious than other subtypes. The team also managed to find two amino acid changes on the S protein region: nt24022 (E1207E) and a non-synonymous alteration in nt25182 (E1207V). However, the sites of mutation appear to be far away from the critical region for ACE2 receptor affinity. Unfortunately, the research does not give out complete data results, and the number of genome data they fetched is also limited.

The effect of variants on clinical morbidity and mortality

Although previous analysis into the specific mutation D614G discovered its strong association with elevated levels of transmission rate, the mutation does not relate to disease severity. While clinical symptoms, as well as the pre-signs, are vital to disease surveillance and control, one specific syndrome is observed to be associated with a higher mortality rate in patients (Velavan et al., 2020). According to Bermejo-Martin's team, lymphopenia and hypercytokinemia, which clinically manifested as uncontrolled progress of the pathogenic invasion, are two main characteristics of a particular immunological phenotype of community-acquired pneumonia (CAP), lymphopenic CAP (L-CAP). The symptoms were also

associated with increased severity, mortality, and a dysregulated immunological response among severe patients (Bermejo-Martin et al., 2020).

While this review features multiple noteworthy and important mutations of SARS-CoV-2 and how they relate to transmission and pathogenicity of the virus by summarizing the findings from published studies, the nature of the published data is also worth paying attention to results derived from epidemiology research or lab work should be interpreted differently depending on factors including research type, research question, etc. Data can be affected by biases, sample size, and quality, and confounding variables, etc. when researchers carry out epidemiological studies. Lab experiments presented should also be watched to assess the quality and accuracy of data.

Limitations

Though this review highlights important information on SARS-CoV-2 mutations and identifies some important variants for future disease surveillance, there are some shortcomings.

First, the number of published reports is still limited, especially regarding the effect of variants on clinical morbidity and mortality. The review is also short of reports from clinical studies, which are crucial to a review studying the subject of the public-health-associated pandemic. Clinical data are fundamentally different from primary research reports where the former give researchers opportunities to compare between clinical cases and multiple groups of control and greater flexibility to manage data based on the study subject they desire to focus on specifically.

Second, although the database resource used by researchers from the reports provides intensive useful information, the reporting may still be limited and lead to skewed analysis. One recommendation is that more countries ought to update and report more genome data to the public database. While identifying mutations and tracking SARS-CoV-2 mutations contribute hugely to improving strategies to tackle coronavirus, researchers can have further motivation and discover more helpful findings by studying the global pattern and the prevalence of different variants with more available, high-quality data worldwide.

Conclusion

In conclusion, several SARS-CoV-2 mutations have been identified and their respective characteristics are discussed. Coming from a public health perspective, some specific variants are also associated with changes in viral transmissibility, clinical symptoms, morbidity, and mortality. Previously identified, notable D614G mutation associated with greater fitness advantage to become a prevalent variant across the globe is one of the highlighted mutations in this review. After the prevalence of the Delta variant, we should also note the new Omicron (B.1.1.529) variant and the greater threat it poses to human health as scientists move the progression of surveilling the newest variant forward. As for the future, the public can also look forward to more promising findings that further identify COVID-19 mutations that are associated with disease severity, greater mortality rate, or the ones that can escape from hosts' immune response, since immune escape can also contribute to higher transmission level overall. The findings summarized in this review can serve as a reference for future advances in developing vaccine strategies and new drug development to combat the COVID-19 pandemic.

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