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GENETIC DIVERSITY ANALYSIS OF THE SARS-CoV-2 VIRUS: A LITERATURE REVIEW

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Keywords: *SARS-CoV-2, COVID-19, genetic monitoring, mutation variants, sequencing, S protein.*

Introduction. *The continuous evolution of the SARS-CoV-2 virus through the accumulation of genetic mutations has led to the emergence of variants with different characteristics, including increased transmissibility, heightened resistance, and changes in disease severity.*

Material and methods. *The research was conducted using open-access international databases such as PubMed, Google Scholar, and national libraries, employing the keywords: SARS-CoV-2, COVID-19, genetic monitoring, mutation variants, sequencing, S protein.*

Results. *Currently, the WHO identifies five major genetic variants of concern (VOCs): Alpha, Beta, Gamma, Delta, and Omicron. The Alpha variant became globally dominant in early 2021 and was replaced by the Delta variant in the summer of 2021. The Delta strain has over 13 mutations, nine of which are found in the S protein. The genome of the Omicron variant contains more than 30 mutations in the conserved domain of the Spike protein. The Omicron variant caused a sharp increase in the number of COVID-19 cases worldwide and was responsible for a record 15 million new infections reported worldwide in one week.*

Conclusions. *The continuous evolution of the SARS-CoV-2 genome poses new challenges for public health. It is essential to study the virus's genetic characteristics to understand mutations, immune evasion, and the persistent effects of infection, with the aim of optimizing prevention and treatment strategies.*

Cuvinte-cheie: *SARS-CoV-2, COVID-19, monitorizarea genetică, variante de mutații, secvențierea, proteina S.*

ANALIZA DIVERSITĂȚII GENETICE A VIRUSULUI SARS-CoV-2: REVIZUIREA LITERATURII

Introducere. *Evoluția continuă a virusului SARS-CoV-2 prin acumularea mutațiilor genetice a generat variante cu diferite caracteristici, inclusiv o transmisibilitate crescută, rezistență sporită și modificări în severitatea bolii.*

Material și metode. *Cercetarea a fost efectuată folosind bazele de date internaționale cu acces deschis – PubMed, Google Academic și bibliotecile naționale, utilizând cuvintele cheie: SARS-CoV-2, COVID-19, monitorizarea genetică, variante de mutații, secvențierea, proteina S.*

Rezultate. *În prezent, OMS identifică cinci variante genetice majore de îngrijorare (VOC): Alpha, Beta, Gamma, Delta și Omicron. Varianta Alpha a devenit dominantă la nivel global la începutul anului 2021 și a fost înlocuită cu varianta Delta din vara anului 2021. Tulpina Delta are mai mult de 13 mutații, dintre care nouă se găsesc în proteina S. Genomul variantei Omicron are mai mult de 30 de mutații în domeniul conservat al proteinei Spike. Varianta Omicron a provocat o creștere bruscă a numărului de cazuri de COVID-19 la nivel mondial și a fost responsabilă pentru un record de 15 milioane de noi infectări raportate în lume în decurs de o săptămână.*

Concluzii. *Evoluția continuă a genomului SARS-CoV-2 impune noi provocări pentru sănătatea publică. Este esențial să se studieze caracteristicile genetice ale virusului pentru a înțelege mutațiile, evaziunea imunității și efectele persistente ale infecției, în vederea optimizării strategiilor de prevenire și tratament.*

INTRODUCTION

At the end of 2019, a new coronavirus subtype, named SARS-CoV-2 (Severe Acute Respiratory Syndrome – Coronavirus 2), was identified as the cause of a cluster of pneumonia cases in Wuhan, China. The virus rapidly spread worldwide, and on March 11, 2020, the World Health Organization (WHO) officially declared the COVID-19 pandemic. The initial impact on public health was devastating, with healthcare systems worldwide overwhelmed by the large number of cases. Travel restrictions, quarantines, and lockdowns became common measures to limit the spread of the virus. In addition to these measures, the pandemic had significant effects on the global economy, disrupting international trade, the workforce, and education, while reshaping the norms of social and professional interactions (1, 2).

The Coronaviridae family, to which SARS-CoV-2 belongs, also includes other epidemiologically significant viruses such as SARS-CoV and MERS-CoV, characterized by a high frequency of genomic alterations (mutations, deletions, recombinations). Since the initial sequencing of its genome in January 2020, SARS-CoV-2 has undergone thousands of unique mutations. Most of these mutations do not affect the virus's virulence or transmissibility, but the most notable ones occur in the Spike protein, particularly in the receptor-binding domain (RBD). These mutations can alter how the RBD binds to the cellular receptor, angiotensin-converting enzyme 2 (ACE2), often increasing the virus's infectivity and leading to the emergence of new genetic variants of SARS-CoV-2 (3 – 6).

The global response to the pandemic included unprecedented international cooperation in research and the rapid development of vaccines, illustrating a new model of global collaboration. However, significant challenges arose, including logistical issues in vaccine distribution, vaccine hesitancy, and disparities in access to medical resources. These aspects highlight the need for continued research to address critical questions left unanswered by the pandemic and to develop strategies to mitigate its effects.

It is important to conduct a literature review for a deeper understanding of the genetic evolution of the SARS-CoV-2 virus and its impact on the COVID-19 pandemic in order to summarize, systematize data, and provide a holistic picture of the current state of knowledge. This can help identify general trends and patterns that might be missed when examining individual publications. These arguments highlight the versatility and importance of conducting a literature analysis to further improve research, practical measures, and response strategies to global health challenges.

The aim of this study was to analyze the existing specialized literature on the genetic characterization and evolution of SARS-CoV-2, with a focus on genomic mutations, the emergence of new variants with epidemiological potential, and their impact on public health.

MATERIAL AND METHODS

For this study, international open-access databases such as PubMed and Google Scholar were used. Source identification was guided by relevant keywords and the following search strategies: *Nidovirales*, deletion 69/70 AND S protein, Concerning Variants AND evolution of SARS-CoV-2, genome characteristic OR genetic monitoring AND COVID-19 AND mutation variants, sequencing AND whole genome AND SARS-CoV-2 virus. To ensure comprehensiveness and relevance of identified materials, additional references were obtained through manual review of bibliographies and citations from initially selected articles. Exclusion criteria: articles published before 2020 to ensure data timeliness, articles lacking a methodology or presenting it unclearly. Inclusion criteria: only relevant articles published in English and Romanian were included to facilitate in-depth analysis. Regarding study types, articles classified as Systematic Review, Prevalence Study, and Qualitative Study were selected to provide a comprehensive overview and robust data analysis.

Therefore, the text of the articles was evaluated based on variables such as the study's aim, methodology, year of completion, and results obtained. From the collected information, the most relevant and recent studies offering clear perspectives on the genetic diversity of the SARS-CoV-2 virus and its impact on public health were selected. These sources were analyzed to highlight emerging trends, significant variations in the virus, and their implications for vaccination and treatment. In this regard, a total of 278 scientific papers were

analyzed, from which, according to selection criteria, 60 publications were analyzed to obtain information about a wide range of genetic characteristics of the COVID-19 causative agent, studying the virus genome mutation mechanisms and the spread of its new strains, of which 15 articles were examined in depth regarding the genetic characterization of VOC variants (fig. 1).

This rigorous methodology enables a comprehensive and systematic evaluation of the literature, essential for understanding the complexity and genetic implications of SARS-CoV-2 in the current global context.

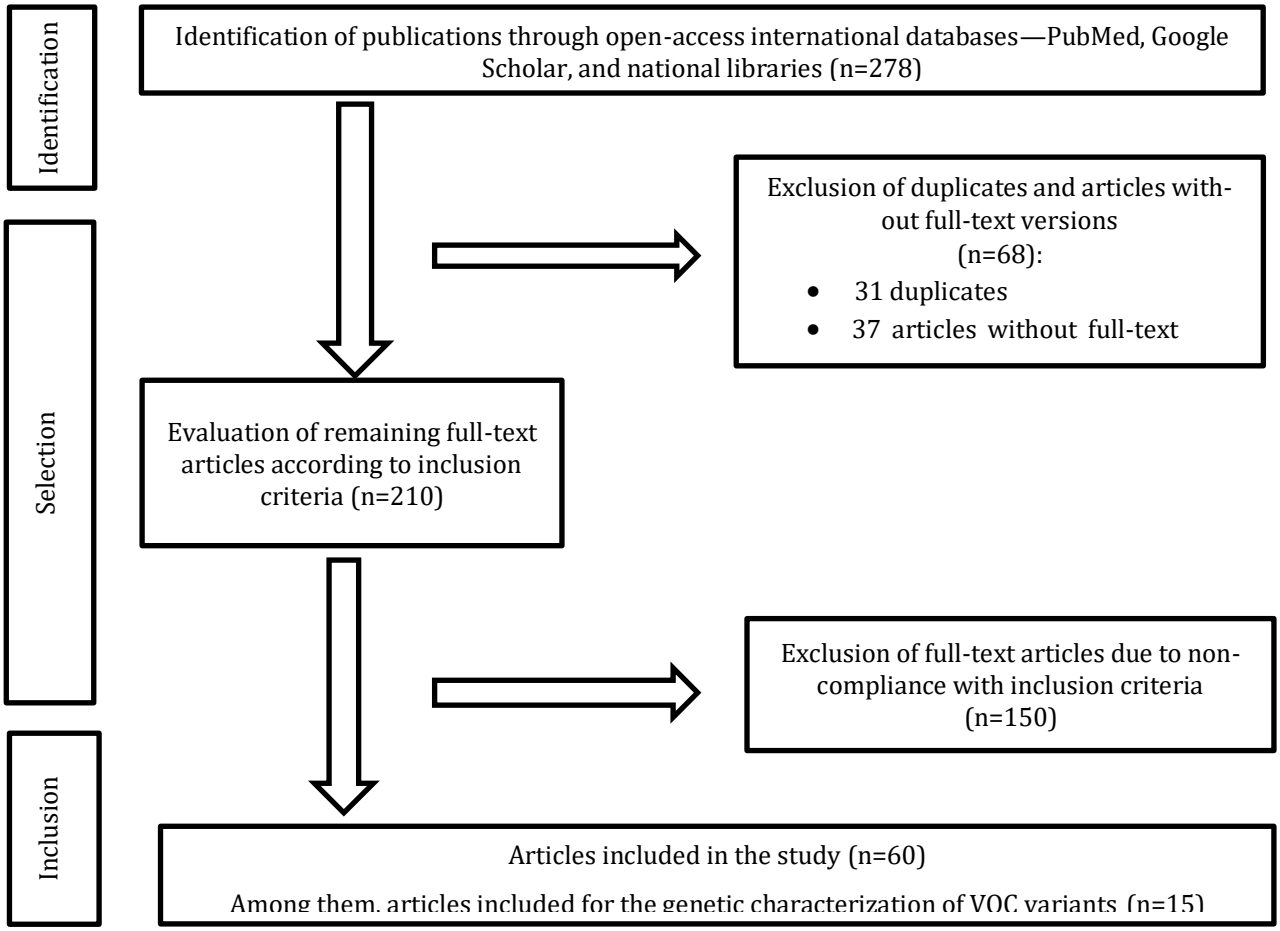

Figure 1. Literature search algorithm.

RESULTS

SARS-CoV-2 is a single-stranded RNA virus, with a genome length of ~29.9 kb. The viral genome consists of six open reading frames (ORFs) common to coronavirus and a series of other accessory genes (7 – 10).

According to phylogenetic analysis, SARS-CoV-2 is more similar to SARS-CoV than to MERS-CoV. It's worth noting that, based on homology modeling studies, SARS-CoV-2 was found to be 96.2% homologous with BatCoV RaTG13, a bat coronavirus from the species *Rhinolophus affinis* (8, 9).

The genome of the SARS-CoV-2 virus encodes 29 proteins, including 16 non-structural proteins (NSP1 – NSP16), necessary for the viral life cycle, 4 structural proteins, and 9 auxiliary protein factors (ORF3a, ORF3b, ORF6, ORF7a, ORF7b, ORF8, ORF9b, ORF9c, and ORF10). The structural N protein, together with viral RNA, forms the virus's nucleocapsid, while the S (Spike), E (envelope), and M (membrane) proteins together form the viral envelope. SARS-CoV-2 has an exceptionally high mutation rate, with numerous mutations – particularly in the Spike gene – correlated with increased transmission rates of SARS-CoV-2, enhanced fusogenic properties, and greater pathogenicity of the virus, as well as the emergence of new variants that could reduce the effectiveness

of existing COVID-19 vaccines and antibody-based therapies. The S protein is responsible for SARS-CoV-2 attachment and entry by binding to host receptors. The ACE2 protein has been identified in various organs, including the respiratory system, gastrointestinal tract, lymph nodes, thymus, bone marrow, spleen, liver, kidneys, and brain, suggesting that the virus has tropism for different organs and tissues. Mutations in the Spike protein, particularly D614G, increase the virus's adaptability by evading vaccine effects, resulting in higher survival and spread rates of the virus. This mutation is found in the S region of the following clades: G/GR/GRY/GH/GV, and it is associated with a high host infection rate due to efficient transmission (11, 12, 13).

Thus, the examined viruses have been scientifically proven to undergo slight and rapid modifications, including their virulence and, to some extent, their antigenic structure, which complicates diagnosis and treatment. Coronaviruses can exhibit genetic variability due to mutations occurring in the viral genome. Genetic mutations are transcription errors of RNA polymerase and can generate new antigenic variants and a limited variation in pathogenic potential (14, 15).

The various clinical presentations in COVID-19 patients are caused by mutations in the SARS-CoV-2 genome. The high frequency of genetic mutations leads to the emergence of new variants, a variation that may explain the differences observed in symptoms and disease severity. Altered ACE2 binding interactions or shifted tissue tropism may arise from a mutation among viral descendants, resulting in aggressive infections (16).

As a result, due to the continuous emergence of multiple SARS-CoV-2 variants, the US Centers for Disease Control and Prevention (CDC) and the World Health Organization (WHO) have independently created classification systems to define emerging viral mutations in several subgroups based on their impact on transmissibility, lethality, and response to therapy. Although there are differences between the two classifications, some alignments exist regarding the first group of variants of concern (VOC), which possess a set of proven characteristics, such as increased infectiousness, severe disease, higher mortality, and a significant reduction in neutralization by antibodies formed in response to previous infection or vaccination. Currently, under WHO classification, the Delta (B.1.617.2) and Omicron (B.1.1.529) variants belong to this category. The second group, variants of interest (VOI), includes variants with specific genetic markers associated with changes in receptor binding, reduced antibody neutralization, increased transmissibility, decreased treatment efficacy, and a potentially anticipated increase in disease severity. The third group consists of variants under monitoring (VUM), for which evidence suggests a potential impact on transmission rates and treatment efficacy, although their prevalence has declined over time to practically zero. There is another category in the CDC classification: variants of high consequence (VOHC), for which there is compelling evidence that existing diagnostic, prevention, and treatment strategies are much less effective than for those that circulated previously (17).

Variant of concern (VOC):

A series of bibliographic sources highlight the characteristics of SARS-CoV-2 mutations related to phenotypic changes compared to the original virus. These mutations span various levels of structural organization, with the most studied being those impacting the sequence level of different viral proteins. One of the first variants reported during the COVID-19 pandemic was D614G in the Spike protein, associated with increased viral load, immune escape, potential drug resistance, and heightened pathogenicity. This amino acid substitution has persisted in the current variants. It has been noted that the region encoding the receptor-binding domain (RBD) of the Spike protein is prone to accumulating changes in SARS-CoV-2; many studies report substitutions along this region, including N501Y, E484K, N439K, S477N, S399P, and K417V. It was hypothesized that changes in this region could alter the binding affinity of SARS-CoV-2 to ACE2. Another reported variant in the Spike protein was P681H, located near the furin cleavage site and associated with increased transmissibility and infectivity of SARS-CoV-2. The main variants of concern exhibit changes in sequences related to the Spike protein, in the RBD and RBM (receptor-binding motif), and at the furin cleavage site (18).

The relevant criteria for inclusion in VOC are: viral mutation variants for which there is evidence of increased transmissibility, more severe disease (e.g., higher rates of hospitalization or mortality), significant reduction in neutralization by antibodies formed during a prior infection or vaccination, reduced treatment effectiveness, vaccine effect-

tiveness, failed diagnostic measures previously performed (compared to earlier variants). Currently, according to WHO, this category includes the Alpha (UK), Beta (South African), Gamma (Brazilian), and Delta (Indian) variants (10, 15).

These SARS-CoV-2 viral mutation variants have captured researchers' interest as strains that, in addition to multiple point mutations, possess more significant mutations driving the virus's evolution toward increased contagiousness, replication capacity, pathogenic potential, and immune response evasion (19 – 23).

Variants of SARS-CoV-2

✓ **Alpha Variant (B.1.1.7)**

The Alpha variant (B.1.1.7), the first strain classified as a VOC, was identified in southeast England in September 2020 and became globally dominant by early 2021. The Alpha variant exhibits a 70% increase in transmissibility due to key modifications, particularly the emergence of the first Spike mutation (D614G), as well as mutations in the RBM (N501Y) and near the furin cleavage site (P681H), which could enhance ACE2 affinity and impact infection development and transmission. This could have contributed to the rapid spread and dominance of this variant worldwide before the emergence of the Delta variant (19, 24, 25, 26, 27, 28).

Marisa A. P. Donnelly and co-authors noted that in December 2020, the Alpha lineage of the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) (a Variant of Concern (VOC), also known as B.1.1.7) was first detected in California and Colorado. By the end of April 2021, Alpha had become the predominant circulating lineage in all regions of the United States and other countries worldwide. Surveillance and modeling suggested that Alpha exhibited increased transmissibility within communities compared to non-Alpha lineages circulating at that time (29).

✓ **Beta Variant (B.1.3.51)**

The Beta strain was first detected in the Nelson Mandela Metropolitan Municipality (South Africa) in October 2020. The Beta variant features the N501Y mutation in the Spike protein, similar to the Alpha strain, along with K417N and E484K mutations, which occur in the binding site. The E484K amino acid substitution helps the virus evade neutralizing antibodies, potentially negatively impacting vaccine efficacy. The E484K spike mutation has been linked to a case of reinfection with the Beta variant of SARS-CoV-2 in Brazil, which researchers believe was the first reinfection case associated with this mutation (30).

Cocherie Théophile and co-authors emphasized that the Beta variant, like the Alpha variant, carries the N501Y substitution, but it is distinct due to the absence of the 69/70 deletion and the presence of E484K and K417N/T substitutions. The K417N/T substitutions impact a neutralizing antibody target epitope in the RBD. These substitutions result in the loss of a salt bridge between the RBD and ACE2 and increase the dissociation constant of their binding. This represents a detrimental mutation in terms of SARS-CoV-2 infectivity, leading to negative natural selection of previously existing strains. This variant was selected due to its ability to evade the humoral immune response developed in the general population, associated with mutations like N501Y or E484K that restore the virus's infective potential. The E484K substitution affects a contact point between the RBD and ACE2, resulting in a conformational rearrangement that tightens the binding interface between the RBD and ACE2 and forms new hydrogen bonds, reducing the dissociation constant. Additionally, the E484K substitution impacts one of the key sites for viral recognition by neutralizing antibodies, lowering their affinity whether derived from vaccines, convalescent plasma, or monoclonal antibody treatments (19, 31).

✓ **Gamma Variant (P.1)**

The Gamma strain (P.1) was first identified on January 6, 2021, by the National Institute of Infectious Diseases (NIID) in Japan in four Japanese individuals returning from the Amazonas state in Brazil. Cowling and Lewi Stone reported that the Gamma variant triggered a second wave of the coronavirus epidemic in Manaus, the capital of Amazonas, despite over 70% of the city's population having developed antibodies following the outbreak in May 2020 caused by another coronavirus variant. The situation was exacerbated by Brazilian President Jair Bolsonaro's opposition to vaccinations, his characterization of the coronavirus as a minor flu, and his disregard for calls to impose a state of emergency or implement strict measures such as mask mandates and self-isolation. The damages inflicted by the new wave of the Gamma strain on residents in tropical regions were so devastating that, at the peak of the out-

break, the healthcare system was overwhelmed and unable to cope with the massive number of patients suffering from severe and critical forms of the disease (32).

The genomic analysis of the Gamma (P.1) SARS-CoV-2 strain revealed that this sublineage exhibits up to 12 mutations in the Spike protein, including N501Y (shared with the Alpha and Beta strains) and E484K (also present in the Beta strain). However, specialists also observed that new mutations appeared in the Gamma variant's genome in the Spike protein K417T, which were not detected in previous strains and which allow the virus to bind more firmly to human cells and, in some cases, evade antibodies. Research has shown that the Gamma strain is three times more contagious than the "Wuhan" coronavirus and is capable of overcoming immunity in those who have recovered from the disease. Moreover, studies indicate that this variant affects young people and pregnant women more severely than other SARS-CoV-2 variants. Furthermore, Mendiola-Pastrana and co-authors highlighted three major genomic alterations in the Gamma (P.1) variant – K417T, E484K, and N501Y – that enhance its affinity for the ACE2 receptor and contribute to an estimated 40% increase in transmissibility compared to earlier variants (33, 34).

✓ **Delta Variant (B.1.617.2)**

The Delta variant (Phylogenetic Assignment of Named Global Outbreak Lineages designated as Pangolin lineage B.1.617.2) of SARS-CoV-2 virus was first detected in India on September 7, 2020. It was classified by the World Health Organization as a variant of concern (VOC) on May 11, 2021, and quickly surpassed other SARS-CoV-2 variants by November 2021, accounting for over 98% of new infections globally (35).

Taylor and co-authors, along with Ong S. and collaborators, noted that this variant exhibits biological and clinical implications, including an increased risk of hospitalization, a longer duration of viral shedding by infected individuals, lower Ct values in PCR tests, higher affinity for the ACE2 receptor, mechanisms to evade the effects of antibodies, and a 50% higher transmissibility rate (19, 36, 37).

The genome of the Delta strain contains more than 13 mutations, nine of which are found in the Spike protein, a surface protrusion of the virus that aids its attachment to human cells. K. Suresh studied the evolution of viruses and identified two mutations located in the receptor-binding domain region, which enable the virus to bind more firmly to cells. The emergence of new mutations in the Delta variant accounted for the increased spread of the virus during the second half of 2021, which significantly declined by December (38).

Kang Min and collaborators emphasized that, compared to the wild-type virus, the Delta variant exhibits 10 specific mutations – T19R, G142D, 156del, 157del, R158G, L452R, T478K, D614G, P681R, and D950N – which may be responsible for its competitive advantages over other variants. The authors noted that the spike mutation of residue 452 located at the receptor-binding domain could enhance immune evasion and resistance to neutralizing antibodies, while P681R in the S1/S2 regions of the S gene could influence proteolytic processing. All these mutations could lead to increased affinity for the ACE2 receptor and resistance to neutralizing antibodies, resulting in increased transmissibility (19).

At the same time, Gomari MM et al. reported that this lineage carries a wide range of mutations, some of which, such as N501Y and P681H, strongly impact the function of the Spike protein. It has been demonstrated that the N501Y substitution in the Spike protein enhances ACE2 binding and cellular infectivity in animal models, while the P681H substitution in the Spike protein affects the furin cleavage site (39).

At the same time, Cocherie T. and collaborators observed that the Delta variant is more competitive than the Alpha variant. In their work, they highlighted that the L452R substitution facilitates the creation of a salt bridge between R454 and D467, which is responsible for conformational changes and a more stable S protein. This conformational change reduces interaction with certain neutralizing antibodies, influencing the cellular immune response and partially blocking the HLA-dependent immune system (MHC class I), thereby contributing to the progression of infection. The T478K substitution results in the replacement of a neutral amino acid with a positively charged basic amino acid at the RBD-ACE2 interface, increasing the interaction strength between RBD and ACE2. Furthermore, it was observed that the combination of L452R and E484Q enhances the RBD-ACE2 binding strength and, consequently, the infectivity of the viral particle. The authors

concluded that this increased transmissibility is associated with heightened virulence. The Delta variant had previously shown fewer symptomatic forms, and an increased risk of severe illness and hospitalization, especially in the unvaccinated populations (35, 40).

✓ **Omicron Variant (B.1.1.529)**

In November 2021, a SARS-CoV-2 variant named Omicron, classified under Pangolin lineage B.1.1.529, was identified in South Africa. Omicron is the most modified SARS-CoV-2 variant, and its near-total transmissibility and immune evasion capabilities have raised global concerns. Due to these characteristics, Omicron rapidly replaced Delta as the dominant variant in multiple regions (41, 42, 43).

This variant features over 30 mutations in the conserved domain of the Spike (S) protein, some of which—69–70del, T95I, G142D/143–145del, K417N, T478K, N501Y, N655Y, N679K, and P681H—are also present in the Alpha, Beta, Gamma, and Delta genome variants, along with 19 mutations in the non-Spike conserved proteins. By the end of January 2022, additional subvariants, BA.2 (B.1.1.529.2) and BA.3 (B.1.1.529.3), were detected in several European countries. The BA.2 subvariant differs from BA.1 in 50 amino acids. Significant differences in amino acid content, particularly in the S protein, between these two subvariants indicate that BA.2 may possess virological characteristics distinct from BA.1. By January 2022, BA.2 had overtaken the BA.1 sublineage in Asia and Europe, suggesting that BA.2 is more infectious due to its enhanced transmission capacity, binding affinity, and immune evasion ability. Since its discovery, the Omicron variant has led to a sharp rise in global COVID-19 cases, accounting for a record 15 million new COVID-19 cases reported worldwide in a single week (39, 44, 45, 46, 47, 48, 49).

The Omicron variant uses the endosomal pathway for cellular penetration. Experimental evidence has shown that Omicron, compared to the Delta variant and the so-called wild-type strain (Wuhan-Hu-1), exhibits a higher tropism for the nasal cavity and bronchial epithelium but a lower tropism for human alveolar cells. Additionally, the Omicron variant is characterized by predominant accelerated replication in the upper respiratory tract and a reduced ability to fuse viral and cellular membranes, which ultimately explains the increased contagiousness of the virus and, at the same time, a milder progression of the disease (39 – 49).

J.A. Lewnard et al. demonstrated that among individuals diagnosed with COVID-19 and monitored in outpatient settings, infection with an Omicron variant was associated with a significantly lower risk of progression to severe clinical forms, including hospitalization, symptomatic hospitalization, intensive care unit admission, mechanical lung ventilation, and mortality, compared to infection with the Delta variant (39 – 49).

Chatterjee S. and co-authors found that the BA.1 sublineage of the Omicron variant was the most widespread globally, but BA.2 progressively replaced BA.1 in many countries, while the transmissibility of BA.3 remained very limited, with the fewest reported cases. Two additional lineages, BA.4 and BA.5, were detected in South Africa in January and February 2022. These sublineages became predominant during the fifth wave of the COVID-19 pandemic that started in South Africa, replacing BA.2, with more than 50% of cases attributed to BA.4 (35%) and BA.5 (20%). Omicron has more mutations than any other variant. These mutations facilitate stronger binding to host cell receptors compared to other reported variants. It also evades most antibodies that block the virus or neutralizing antibodies produced by vaccinated individuals or individuals infected with other variants (43, 50).

Subsequently, Tamura T. and co-authors described that the BQ.1.1 sublineage of the Omicron variant, a descendant of BA.5, became predominant in Western countries in December 2022. The authors noted that this sublineage contains all the convergent substitutions, such as R346T, K444T, L452R, N460K, and F486V, which enhance the binding affinity of the SARS-CoV-2 S protein to the human angiotensin-converting enzyme 2 (ACE2) while also contributing to the evasion of humoral antiviral immunity induced by vaccination and natural SARS-CoV-2 infection. Furthermore, due to ongoing mutations in the Omicron variant genome, a recombinant variant named XBB emerged. The Omicron XBB variant likely arose from the recombination of two BA.2 descendants, BJ.1 and BM.1.1.1, as well as a descendant of BA.2.7516. While the BQ.1 lineage became dominant in Europe, XBB established dominance in In-

dia and Singapore. Subsequently, on October 28, 2022, the WHO classified XBB as a subvariant of Omicron under monitoring (43, 51).

In the article presented by Dinah V. Parums, a detailed study is described regarding the new sublineage of the Omicron variant, EG.5 (Eris), which was first recorded by the World Health Organization on February 17, 2023, and designated as a variant under monitoring (VUM) on July 19, 2023. This sublineage and its derivatives – EG.5.1, EG.5.1.1, and EG.5.2 – are descendants of XBB.1.9.2, sharing the same amino acid spike profile as XBB.1.5 (Kraken). However, the authors noted that EG.5 (Eris) includes an additional amino acid mutation, F456L, in the Spike protein compared to its parental subvariants, while the EG.5.1 subvariant contains another Spike mutation, Q52H. Subsequently, following WHO risk assessments on August 8, 2023, EG.5 (Eris) and its sublineages were designated as a variant of interest (VOI) (25, 43, 52).

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has caused millions of deaths and substantial morbidity worldwide since 2019. The intensive scientific effort to understand the biology of SARS-CoV-2 has resulted in a daunting number of genomic sequences. We have witnessed evolutionary phenomena, including the emergence of variants with distinct phenotypes, such as transmissibility, severity, and immune evasion (53).

Currently, this virus has attained the status of the most studied virus: during the pandemic, an enormous amount of genomic sequences was obtained, providing extensive information about this pathogen, which has enabled not only the development of strategies to combat it but also a deeper understanding of its biology, characteristics, and mutation dynamics. Each new variant or sublineage of the virus has been and continues to be studied in detail. Moreover, while initially, many believed that the virus would become more transmissible but less virulent as mutations accumulated, in reality, the new sublineages of SARS-CoV-2 mutate very rapidly, gaining an increasing number of opportunities to evade all forms of immunity, penetrate more easily into human cells, and continue to cause severe damage.

Markov P.V. et al. predict that as the Omicron variant continues to circulate, immunity induced by a combination of vaccination and prior infection will provide protection against severe illness upon reinfection. However, an alternative scenario exists: a new variant could emerge with a completely different set of mutations and properties, allowing the virus to evade immunity created by prior infections or vaccines. This could result from the accelerated evolution of the virus during long-term persistence in immunocompromised individuals. At the same time, predicting the virulence of new strains is challenging; it is quite possible that variants may arise that cause severe disease in more people than the Omicron variant (54, 55).

DISCUSSIONS

Compared to their hosts, RNA viruses have mutation rates that can be over a million times higher due to their limited ability to correct replication errors (56, 57, 58, 59, 60). The survival and adaptability of RNA viruses depend on their ability to overcome harmful mutations and evolve into forms that provide competitive advantages, such as better adaptation to their hosts. SARS-CoV-2 undergoes genetic evolution as it adapts to its new human hosts, resulting in mutations in the viral genome that can potentially modify the virus's pathogenic potential.

Based on the analysis of scientific publications, it was established that from the early days of the COVID-19 pandemic, multiple research directions were initiated, including a detailed examination of the SARS-CoV-2 causative agent and its mutations over time and space (tab. 1). This information is extremely important because it helps understand the mechanisms of pathogenesis and resistance of the causative agent and the actual mutation directions, which have been observed since the early days of the pandemic. The genomic changes of the Alpha variant (B.1.1.7) were linked to mutations in seven genes and some proteins, but these were likely neutral concerning protein function (1, 2 in tab. 1). It was assumed that the observed mutations influence the clinical presentation and disease severity (3, 4 in tab. 1). Examining the genomic characterization of the Beta variant (B.1.3.51), it was found that mutations in the RBD region led to the loss of the salt bridge between RBD and ACE2, thus increasing their dissociation con stant. Amino acid metabolism modification contributes to the increased viral nucleic acid quantity in the upper respiratory tract (5, 6 in tab. 1).

Table 1. Analysis of articles regarding the genetic characteristics of VOC variants.

Nr.	Authors	Content of the article	Year of publication
Genomic characteristics of the Alpha variant (B.1.1.7)			
1.	A. Nagy, S. Pongor, B. Gyorffya	Mutations in seven genes: L54F, D614G, and V1176F in the spike glycoprotein (S), A97V and P323L in RNA polymerase, Q57H and G251V in ORF3a, P13L, S194L, R203K, G204R, and I292T in the nucleocapsid phosphoprotein, I33T in the ORF6 protein, and mutations S1197R and T1198K in the NSP3 protein.	2021
2.	S Isabel, L. Grana-Miraglia, J. M. Gutierrez et al.,	Analysis of over 1,225 SARS-CoV-2 genomes from December 2019 to March 2020 revealed the presence of the D614G missense mutation in the SARS-CoV-2 Spike protein. The authors suggested that the mutation is most likely neutral concerning the protein's function.	2020
3.	C. P. Morris, Chun Huai Luo, A. A. M. Schwartz et al.	It was assumed that the N501Y mutation in variants B.1.1.7, P1, and B.1.351 could affect ACE2 binding and have an impact on disease severity.	2021
4.	R. A. Mansbach, S.Chakraborty, Kien Nguyen et al.	The amino acid substitution in Spike at residue 614 from aspartic acid (D) to glycine (G) (D614G) is more transmissible and contributes to an increase in viral nucleic acid quantity in the upper respiratory tract.	2021
Genomic characteristics of the Beta variant (B.1.3.51)			
5.	Cocherie T., Zafilaza K., Leducq, V. et al.	The K417N/T substitutions affect a neutralizing antibody target epitope of the RBD, leading to the loss of a salt bridge between the RBD and ACE2 and an increased dissociation constant of their binding.	2022
6.	Cele S., Gazy I., Jackson L. et al.	The E484 substitution is a binding site for highly potent neutralizing antibodies. Mutations causing substitutions at E484 have emerged as immune escape mutations and have conferred broad cross-resistance to monoclonal antibody panels and plasma neutralization from convalescent individuals.	2021
Genomic characteristics of the Gamma variant (P.1)			
7.	Mendiola-Pastrana IR., Lopez-Ortiz E., Rio de la Loza-Zamora J.G. et al.	The Gamma strain (P.1) exhibits three major modifications in the viral genome—K417T, E484K, and N501Y—that confer affinity for the ACE2 receptor and contribute to an estimated 40% increase in transmissibility compared to the earlier variants.	2022
8.	Paul M., Chun Huai Luo, A. Amadi et al.	The genomes of the Beta (B.1.351) and Gamma (P.1) variants display mutations in the S protein: N501Y and E484K, which increase the binding affinity to angiotensin-converting enzyme 2 (ACE2).	Iulie 2021
9.	M. Gomari, P. Tarighi, E. Choupani et al.	The genome of the P.1 variant contains the N501Y, E484K, and K417T substitutions, the most important amino acid mutations, which can induce conformational changes in the Spike protein.	2023
Genomic characteristics of the Delta variant (B.1.617.2)			
10.	Min Kang, Hualei Xin, Jun Yuan et al.	Compared to the wild-type virus, the Delta variant has nine or ten characteristic mutations: T19R, G142D, 156del, 157del, R158G, L452R, T478K, D614G, P681R, and D950N.	2022
11.	M. M. Gomari, P. Tarighi, E. Choupani et al.	The Delta variant has a number of mutations, some of which, N501Y and P681H, affect the function of the Spike protein. The N501Y substitution enhances the binding of the Spike protein to ACE2, while the P681H substitution affects the furin cleavage site.	2023
12.	Th.Cocherie, K. Zafilaza, V. Leducq et al.	The L452R substitution affects the RBD region and leads to the formation of a salt bridge between R454 and D467, responsible for a conformational change, which impacts the cellular immune response by modifying the 448-456 region of the S protein.	2023

Nr.	Authors	Content of the article	Year of publication
Genomic characteristics of the Omicron variant (B.1.1.529)			
13.	D. Setiabudi, Y.Sribudiani, K. Hermawan et al.	This variant has more than thirty mutations in the conserved region of the Spike protein (S), including 69–70del, T95I, G142D/143–145del, K417N, T478K, N501Y, N655Y, N679K, and P681H, which overlap with those found in the Alpha, Beta, Gamma, or Delta variants.	2022
14.	Sh. Pather, Sh.A. Madhi, B.J.Couling et al.	Essential mutations of different sublines of the Omicron variant with biological significance: del69–70, G142D, del143–145, R346K, S371L, N440K, G446S - Resistance to neutralizing antibodies; L452R, L452Q, N501Y - Increased binding to ACE2; F486V, E484A – Escape from neutralizing antibodies; Q493R - Increased binding to ACE2 and escape from neutralizing antibodies; H655Y, P681H - Increased binding to ACE2, transmissibility, and enhanced endosomal entry; N969K - Reduced fusogenicity in the S2 domain; del3674–3676, del3675–3677 - Protein stability; del27–29 - Suppression of immune response.	2023
15	Fan. Y, Asao. S, Furbank. R et al.	BA.1 and BA.2 have 12 mutations in the RBD, including G339D, S373P, S375F, K417N, N440K, S477N, T478K, E484A, Q493R, Q498R, N501Y, and Y505H. S371L, G446S, and G496S were identified only in BA.1, while R346K was found in one member of this group, namely BA.1.1. BA.2 possesses two unique mutations in the RBD, including S371F and R408S, and shares T376A and D405N with BA.3. The authors state that some of these mutations were also found in previous variants.	2022

Examining the genome of the Gamma variant (P.1), changes were observed that contribute to the increased transmissibility, estimated to be 40% higher compared to earlier SARS-CoV-2 variants, and induce conformational changes in the Spike protein (7-9 in tab. 1). Compared to the wild-type virus, the Delta variant has up to ten characteristic mutations, some of which affect the Spike protein function and other proteins, impacting the cellular immune response (10-12 in tab. 1). It was found that the genome of the Omicron variant has more than thirty mutations in the conserved domain of the Spike protein, including 69–70del, T95I, G142D/143–145del, K417N, T478K, N501Y, N655Y, N679K, and P681H, which overlap with those found in the Alpha, Beta, Gamma, or Delta variants. Importantly, these mutations trigger protective mechanisms, immune response suppression, and antibody formation (13-15 in tab. 1).

Peter V. Markov and coauthors offer hope of understanding the processes that generate this diversity, predicting the possible future evolution ary trajectories of the virus, and developing means of prevention and treatment. To facilitate such possibilities, there is an urgent need to critically review the key factors of SARS-CoV-2 evolution and to explain the processes that generate diversity and novelty in the virus (53).

In this context, it is important to understand the mechanisms that generate genetic variations in SARS-CoV-2, which underlie processes within the host and at the population level. To understand how major lineages, such as variants of concern (VOC), are generated, bibliographic data have been analyzed and included in five tables according to the number of SARS-CoV-2 variants.

Studying the genetic characteristics of the SARS-CoV-2 virus provides valuable opportunities for the development of more effective treatments, the adaptation of diagnostic tests, and the next generations of vaccines. Additionally, understanding the dynamics and molecular evolution mechanisms of different mutated variants is crucial for anticipating and combating the pandemic spread.

CONCLUSIONS

1. Coronaviruses are RNA viruses with large genomes, which give them persistence in a variety of hosts and environments.
2. SARS-CoV-2 has the ability to adapt and coexist long-term in the human population.

3. The continuous evolution of the SARS-CoV-2 genome has generated new variants with significant public health impacts.
4. Studies on mutations and persistent effects are essential, and collaboration between researchers, authorities, and the pharmaceutical industry is crucial for the prevention of COVID-19 and the development of therapy.

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