Check for updates

journal of clinical practice 2021 Vol 12 №1

GENETICS OF COVID-19

© D.A. Vologzhanin[1, 2], A.S. Golota[1], T.A. Kamilova[1], O. V. Shneider[1], S.G. Sherbak[1, 2]

[1] Saint-Petersburg City Hospital No 40 of Kurortny District, Saint Petersburg, Russian Federation

[2] Saint-Petersburg State University, Saint Petersburg, Russian Federation

COVID-19 is characterized by a wide range of clinical manifestations, from asymptomatic to extremely severe. At the onset of the pandemic, it became clear that old age and chronic illness were the major risk factors. However, they do not fully explain the variety of symptoms and complications of the SARS-COV-2 coronavirus infection. The research on genetic risk factors for COVID-19 is still at its early stages. A number of mutations and polymorphisms have been identified that affect the structure and stability of proteins — factors of susceptibility to SARS-COV-2 infection, as well as a predisposition to the development of respiratory failure and the need for intensive care. Most of the identified genetic factors are related to the function of the immune system. On the other hand, the genetic polymorphism of the virus itself affects the COVID-19 spread and severity of its course . The genome of the virus accumulates mutations and evolves towards increasing contagiousness, replicative ability and evasion from the host's immune system. Genetic determinants of the COVID-19 infection are potential therapeutic targets. Studying them will provide information for the development of drugs and vaccines to combat the pandemic.

Keywords: *COVID-19; coronavirus; SARS-COV-2; genetic predisposition factors; mutation; polymorphism.*

For citation: Golota AS, Vologzhanin DA, Kamilova TA, Shneider OV, Sherbak SG. Genetics of COVID-19. *Journal of Clinical Practice.* 2021;12(1):41–52. doi: 10.17816/clinpract64972

Submitted 19.02.2021 Revised 25.02.2021 Published 01.04.2021

BACKGROUND

On March 11, 2020, the World Health Organization declared COVID-19 pandemic. During the COVID-19 pandemic, 132,046,206 people worldwide were infected with the SARS-CoV-2 coronavirus (as of 04/07/2021) with a registered mortality of 2,867,242 people [1]. In most cases, patients infected with the SARS-CoV-2 coronavirus have mild or asymptomatic course, while 5% of COVID-19 patients develop pneumonia, acute respiratory distress syndrome, septic shock, and multiple organ failure, which often result in lethal outcome [2, 3].

HUMAN GENETICS

Severe COVID-19 represents a range of hyperinflammatory, often fatal conditions. Susceptibility to life-threatening infections and immune-mediated diseases has a genetic component. In particular, susceptibility to respiratory viruses such as influenza is inherited and associated with specific genetic variants [4]. Revealing the molecular genetic mechanisms of this variability is of primary biological and medical importance [5]. The determinants of COVID-19 severity are almost entirely dependent on host factors and not on the virus [6].

D. Ellinghaus and other members of the Severe COVID-19 GWAS Research Group from Germany, Sweden, Norway, Italy, Spain, Australia, and Lithuania performed a meta-analysis of genome-wide association studies (GWAS) in cohorts of patients with severe COVID-19 (defined as respiratory failure), hospitalized in 7 hospitals in Italian and Spanish epicenters of the local peak of the epidemic, which received oxygen therapy or artificial pulmonary ventilation (APV), and compared the data of these patients with data from healthy blood donors from the same regions [7]. The final analysis included 835 patients and 1255 control participants from Italy, as well as 775 patients and 950 control participants from Spain. A total of 8,582,968 single nucleotide polymorphisms (SNPs) were analyzed. The study revealed associations of the severity of SARS-CoV-2 infection with polymorphism of polygenic loci 3p21.31 and 9q34.2. Cross-reproducible associations were found with the variants rs11385942 (GA insertion/deletion) at the 3p21.31 locus and rs657152 (CA SNP) at the 9q34.2 locus (both $p < 5 \times 10^{-8}$). At the 3p21.31 locus, the association encompassed the genes *SLC6A20*, *LZTFL1*, *CCR9*, *FYCO1*, *CXCR6*, and *XCR1*, and at the 9q34.2 locus, the association signal coincided with the ABO blood group locus as an increased risk in blood group A ($p = 1.5 \times 10^{-4}$) and a protective effect in blood group O ($p = 1.1 \times 10^{-5}$) compared with other blood groups. Correction for

gender and age confirmed the associations for sites rs11385942 (OR 2.11; $p = 9.46 \times 10^{-12}$) and rs657152 (OR 1.39; $p = 5.35 \times 10^{-7}$). The biological mechanism underlying the effect of the rs657152 polymorphism at the ABO locus is presumably related to the production of neutralizing antibodies against viral proteins [7]. Meta-analysis showed that it is associated with susceptibility to COVID-19, but not with the severity of the disease [8].

Among the six candidate genes at the 3p21.31 locus, the *LZTFL1* gene with the rs11385942 variant is the most convincing, which is expressed at a high level in human lung cells and encodes the protein involved in the transport of proteins to primary cilia which are subcellular organelles from microtubules that function as antennas-mechanosensors for extracellular signals. In the main meta-analysis and gender- and age-adjusted meta-analyzes, the frequency of the risk G allele at site rs11385942 is higher in patients who used APV than in those receiving oxygen supplementation alone. In addition, patients homozygous for the risk allele were younger than heterozygous or homozygous for the A allele (mean age 59 and 66 years, respectively; $p = 0.005$) [7]. In T-lymphocytes, the LZTFL1 protein participates in immunological synapse with antigen-presenting cells. The 3p21.31 locus contains the *SLC6A20* gene, which encodes a high intestinal expression transporter protein regulated by the ACE2 receptor, and genes encoding chemokine receptors, including *CXCR6*, which regulates T cell migration and the localization of CD8+ resident memory T cells in the lungs. The *CCR9, XCR1*, and *FYCO1* genes are also involved in the function of dendritic and T cells [6]. Thus, the 3p21.31 gene cluster has been identified as a locus of genetic predisposition to the most severe forms of COVID-19.

Researchers from 86 clinics and laboratories in America, Europe, Asia, and Australia sequenced the exome or genome of 659 patients with severe COVID-19-associated pneumonia and 534 patients with asymptomatic or mild infection and revealed a significant increase in the number of loss-of-function mutations at 13 candidate loci in patients with life-threatening pneumonia compared with patients with asymptomatic or mild course. In 3.5% of patients aged 17 to 77 years, 24 pathogenic variants were identified, that predetermine autosomal recessive defects in the *IRF7* (interferon regulatory factor 7) and *IFNAR1* (interferon alpha/beta receptor alpha chain) genes and autosomal dominant defects in the genes *TLR3*, *UNC93B1*, *TICAM1*, *TBK1*, *IRF3*, *IRF7*, *IFNAR1*, and *IFNAR2* involved in TLR3- and IRF7-dependent induction and amplification of type I IFN. The *IFNAR1* and *IFNAR2* genes are part of a cluster of immunologically important genes and encode subunits 1 and 2 of the receptor IFN-α and IFN-β, respectively, involved in the pathophysiology of severe COVID-19. Plasmacytoid dendritic cells of patients with IRF7 deficiency do not produce type I IFN when infected with SARS-CoV-2. Fibroblasts from patients with the $TLR3^{-/-}$, $TLR3^{+/-}$, $IRF7^{-/-}$, and $IFNAR1^{-/-}$ phenotypes are susceptible to SARS-CoV-2 infection *in vitro*. These data reveal the role of the TLR3 receptor as a sensor for double-stranded RNA and type I IFN as an element of innate cellular immunity in the control of SARS-CoV-2 infection. The introduction of exogenous type I IFN may have a therapeutic effect in COVID-19 patients who are carriers of a certain genotype [5].

Understanding the role of circulating proteins in infectious diseases is challenging since the infection itself often alters significantly the circulating protein expression and it might appear that increased levels of circulating proteins, such as cytokines, are associated with poorer outcome, while in fact it may be a host response to infection and helps mitigate this outcome. That is why it is important to know the genetic determinants of protein levels, which reflect the degree of a person's protection against severe COVID-19. A large-scale randomized study [9] conducted in the USA, Canada, Japan, Sweden, Germany, and England to search for circulating proteins that influence the susceptibility and severity of COVID-19, identified the *OAS1* gene associated with a decrease in susceptibility to COVID-19 (14,134 patients and 1,284,876 controls; $p = 8 \times 10^{-6}$), hospitalizations with COVID-19 (6,406 patients and 902,088 controls; $p = 8 \times 10^{-8}$), and mortality from COVID-19 (4,336 patients and 623,902 controls, $p = 7 \times 10^{-8}$). By measuring the expression of circulating proteins, the authors demonstrated that this protective effect on the outcome of COVID-19 is provided by increased levels of the p46 OAS1 isoform and total OAS1 protein, which is consistent with the findings of H. Zeberg et al. [10].

OAS proteins are part of the innate immune response against RNA-viruses. They activate latent RNase L which cleaves double-stranded RNA, an intermediate link in the coronavirus replication, leading to direct destruction of viral RNA. SARS-CoV-2 and other beta coronaviruses produce viral proteins that destroy OAS enzymes and counteract RNase L which degrades viral RNA. This viral activity allows it to evade the host's immune response. Inhibitors of viral phosphodiesterase-12, which degrades OAS enzymes,

https://doi.org/10.17816/clinpract64972

journal of clinical practice 2021 Vol 12 №1

also enhance the antiviral activity of OAS. Protective isoforms of proteins OAS1, OAS2, and OAS3 increase the expression of genes *IRF3* and *IRF7* included in the interferon-inducible gene signature. OAS1 polymorphisms are associated with the host's immune response to viral infections, including influenza, herpes simplex, hepatitis C, dengue, SARS-CoV, and SARS-CoV-2 viruses. Given that OAS1 is an intracellular viral RNA degradation enzyme, the circulating levels of this enzyme reflect probably its intracellular levels. Both intracellular and circulating OAS1 are significant for the antiviral immune response [9].

GWAS of 2244 patients with severe COVID-19 with deep hypoxemic respiratory failure from 208 British hospitals confirmed significant associations of the disease severity with a number of polymorphisms related to key mechanisms of host antiviral defense and mediators of inflammatory organ damage in COVID-19, namely rs10735079 ($p = 1.65 \times 10^{-8}$), rs2109069 ($p = 3.98 \times 10^{-12}$), rs2236757 ($p = 4.99 \times 10^{-8}$), rs74956615 ($p = 2.3 \times 10^{-8}$). The rs10735079 variant is located in the OAS (oligoadenylate synthetase, locus 12q24.13) gene cluster encoding interferon-inducible activators of restriction enzymes of antiviral protection OAS1, OAS2, and OAS3. Transcriptome analysis of lung tissue revealed a significant association of COVID-19 with OAS3 expression. High levels of OAS3 in lungs and whole blood are associated with worse outcomes in severely ill COVID-19 patients, which is the opposite directional effect compared to OAS1 [8].

To study a haplotype that is protective against severe COVID-19 in the abovementioned OAS gene cluster on chromosome 12, H. Zeberg et al. [11] used the Genetics of Mortality in Critical Care and COVID-19 Host Genetics Initiative databases. This haplotype contains the variants rs2660, rs1859330, rs1859329, rs2285932, and rs1293767 [11]. In addition, protective alleles rs4767027-T and rs10774671-G were found in the OAS1 gene. Alternative splicing of *OAS1*, regulated by the rs10774671-G allele, increases the expression of the p46 isoform which has a higher antiviral activity than the p42 isoform. Host genetic variants associated with extremely severe disease help identify the therapeutic targets. Molecules are already known that can increase the activity of OAS1. Interferon β-1b (IFN-β1b), which activates the cytokine cascade leading to an increase in the expression of the *OAS1* gene, increases the level of OAS1 in the blood. IFN-β1b inhalation therapy can have different effects in populations of different origins due to the presence of different genetic variants, in particular, it is more effective in populations with a higher expression of the p46 isoform [9].

The rs2109069 variant in the *DPP9* gene (dipeptidyl peptidase 9, locus 19p13.3) is associated with idiopathic pulmonary fibrosis. Serine protease DPP9 is significant in antigenic presentation and activation of inflammation. The *IFNAR2* gene (locus 21q22.1), which contains the rs2236757 variant, encodes an interferon receptor involved in type 1 interferon signaling. The rs74956615 variant is located near the *TYK2* (tyrosine kinase 2) gene on chromosome 19, the expression of which is associated with an extremely severe form of COVID-19. *TYK2* is one of the target genes for inhibitors of the JAK/STAT signaling pathway, such as baricitinib [8].

Some of the genetic associations with severe COVID-19 relate to the immune-mediated phase of the disease, associated with respiratory failure and requiring invasive mechanical ventilation. The extremely severe course of COVID-19 is associated with at least two biological mechanisms, namely innate antiviral protection, which is especially important at an early stage of the disease (*IFNAR2* and *OAS* genes), and inflammatory lung disease, which s a key mechanism of the late phase of COVID-19 (*DPP9, TYK2*, and *CCR2* genes). Interferons are mediators of the transmission of antiviral signals and stimulate the release of components of an early response to viral infection. Increased expression of the IFNAR2 interferon receptor subunit, which is consistent with the protective role of type I interferons, reduces the probability of severe COVID-19. Loss of function mutations in the *IFNAR2* gene are associated with severe COVID-19 [5] and other viral infections. The administration of interferon may reduce the probability of a critical condition in COVID-19, but it has not been determined at what term of the disease the treatment will be effective. Treatment with exogenous interferon did not reduce the mortality of hospitalized patients in large-scale clinical trials [12]; this genetic effect acts probably at an early stage of the disease, when the viral load is high [8].

The extrapulmonary effects of COVID-19 can be mediated by an IFN-controlled increase in ACE2 expression on both endothelial and parenchymal cells, which leads to endotheliitis [13] and liver damage in 60% of patients with severe course [14]. Immunity deficiency mediated by IFN type I is associated with life-threatening COVID-19 pneumonia [5], and induction of gene signatures by type I interferons is found in some critically ill patients. Metatranscriptome sequencing for profiling immune signatures in bronchoalveolar lavage fluid in 8 cases of COVID-19 showed that the expression of 83 pro-inflammatory genes, es-

pecially those encoding cytokines (*IL1RN* and *ILB*) and chemokines (*CXCL17, CXCL8*, and *CCL2*), as well as CXCR2 for chemokines CXCL8, CXCL1, and CXCL2, was noticeably increased in COVID-19 cases compared to patients with community-acquired pneumonia and healthy controls, indicating hypercytokinemia in COVID-19 patients caused by the expression of multiple IFN-stimulated genes (ISG). Among ISGs, genes with immunopathogenic potential involved in inflammation predominate. Transcriptome data were also used to assess populations of immune cells, detecting an increase in activated dendritic cells and neutrophils. Activation of genes *IL1RN* and *SOCS3*, which encode antagonists of cytokine signaling, suggests that SARS-CoV-2 infection involves a negative feedback loop. The expression of genes involved in the morphogenesis and migration of immune cells (*NCKAP1L*, *DOCK2*, *SPN*, and *DOCK10*) was found to be lower than in healthy controls. The functional analysis revealed a state of high sensitivity to noxious stimuli in cases of COVID-19, characterized by powerful defensive reactions and hyperactive ribosome biogenesis. The study of changes of cytokine expression over time showed that the expression levels of genes associated with cytokines decrease with time. The patient who eventually deceased turned out to be an exception. These cases showed that severe inflammation in COVID-19 gradually resolves, and unresolved inflammation can lead to fatal consequences [15].

The research results suggest that type I IFNs play a bivalent role in the pathobiology of COVID-19, which requires heavy regulation, and lead to the hypothesis that JAK/STAT inhibitors are useful early in the disease by reducing IFN-I-induced expression of ACE2. Attention should be paid to important qualitative differences between the response of liver spheroids, where IFNs induced ACE2 and increased infectivity, and lung organelles, where IFN signaling did not affect ACE2 and viral load. Vascular endothelial cells express high levels of ACE2 [16] and are very sensitive to IFN signaling [17]. Taken together, these data suggest that the effects of baricitinib may differ in different organ systems and that anti-inflammatory effects may be most beneficial in those tissues in which *ACE2* gene expression is a response to IFN, including the liver [18].

Genotyping of 322,948 biological samples from the UKB biobank for the *ApoE* gene (apolipoprotein E) established that homozygotes *ApoE* e4e4 (n = 9022; 3%) were more likely to have a positive test result for COVID-19 (OR 2.31; $p = 1.19 \times 10^{-6}$) compared to e3e3 homozygotes (the most common genotype, n = 223,457, 69%). This association persisted after excluding samples of patients with diseases associated with the severity of COVID-19 (hypertension, coronary heart disease, myocardial infarction, angina pectoris, diabetes, dementia) from the analysis. Therefore, it is a fair assumption to say that the e4 allele of the *ApoE* gene, a variant associated with an increased risk of Alzheimer's disease, increases the risk of severe COVID-19 infection regardless of other risk factors. *ApoE* is one of the highly expressed genes in type II alveolar lung cells. The *ApoE* e4 variant affects not only the function of lipoproteins and the development of cardiometabolic diseases, but also the pro/anti-inflammatory phenotypes of macrophages. Further research is required to understand the biological mechanisms linking the *ApoE* genotypes to the severity of COVID-19 [19].

The fact that men are more at risk of severe COVID-19 is due in part to the localization of the *ACE2* gene on the X chromosome [20]. In the region covering the entire *ACE2* gene and 50,000 base pairs around it, SNPs have been found, that carry alleles inherited from Neanderthalers. These SNPs are not in linkage disequilibrium and therefore do not form a continuous haplotype. Neanderthal haplotypes in the *DPP4* gene (*DPP9* homologue) are associated with an approximately 80% increased risk of hospitalization after infection with SARS-CoV-2. The S-protein of SARS-CoV-2 binds to the membrane-bound DPP4 receptor (known as CD26) [21]. DPP4 is involved in several physiological systems, including the regulation of glucose metabolism. DPP4 inhibitors are used to treat diabetes and are thought to influence COVID-19 outcomes [22]. However, the Neanderthal variant of the *DPP4* gene doubles the risk of severe COVID-19 course [23]. The strongest association with severe COVID-19 was found in SNP of rs117888248 (OR 1.84). Neanderthal haplotypes in the *DPP4* gene and on chromosome 3 increase the risk of severe COVID-19 with respiratory failure and the need for APV by 100% each. Both risk haplotypes in the *ACE2* and *DPP4* genes have stronger effects than the protective Neanderthal haplotype on chromosome 12, which reduces the risk of severe disease by approximately 23% [11].

The Neanderthal variant of the *DPP4* gene is present in 1% of Europeans, 2.5% of South Asians, 4% of East Asians, and 0.7% of Americans. Three currently available genomes of Neanderthalers from Europe and southern Siberia, whose age ranges from 50 to 120 thousand years, are homozygous for risk variants. This means that if a Neanderthal were alive today, they

https://doi.org/10.17816/clinpract64972

journal of clinical practice 2021 Vol 12 №1

would have a 4–16 times higher risk of severe illness if infected with the SARS-CoV-2 virus [23].

Advances in proteomics, combined with human genetic data, contribute to identification of therapeutic targets and development of drugs against COVID-19. Identifying a causal relationship between circulating proteins and susceptibility to SARS-CoV-2 infection or the severity of COVID-19 is a promising field in the development of pharmacotherapy for this disease, in which exposure to SARS-CoV-2 causes profound changes in the levels of circulating proteins. Several genetic associations lead to potential therapeutic approaches for enhancing interferon signaling, counteracting the activation and infiltration of leukocytes into the lungs, or targeting specifically the inflammatory pathways [8].

GENETICS OF THE SARS-CoV-2 CORONAVIRUS

Genomes of 5,085 SARS-CoV-2 strains (1,026 strains belonging to the earliest confirmed cases of COVID-19, and 4059 strains obtained during the massive resurgence 2 of the pandemic) have been sequenced in a large metropolis (Houston, USA) which is an ethnically diverse region with 7 million residents. Analysis of the SARS-CoV-2 strains that caused the disease in the resurgence 1 (05.03–11.05.2020) revealed a wide variety of viral genomes, which represent together the main monophylogenetic groups identified in the world today, although not all "branches" of the evolutionary tree SARS-CoV-2 is represented in this data. The phylogenetic distribution of strains with multiple substitutions of different amino acids at the same site showed their independent origin. Almost all strains (4054) of the resurgence 2 have a substitution of the amino acid asparagine-614 in the receptor binding domain (RBD) of the S-protein for glycine, which is associated with increased transmissibility and infectivity. Strains with the Gly614 variant in the S-protein accounted for 71% of SARS-CoV-2 strains at the beginning of the resurgence 1 and 99.9% in the resurgence 2 ($p < 0.0001$). Patients infected with Gly614 strains had significantly higher viral load at the upper respiratory tract at initial diagnosis than patients infected with Asp614 variant strains. At the same time, the association of the disease severity with concomitant diseases and human genetics persists. The presence of the Gly614 variant did not correlate with the disease outcome. Certain regions of the S protein as a major target of global efforts in creating the vaccine abound with amino acid substitutions, possibly indicating the selection effect. In the RBD of the S protein, amino acid substitutions are relatively rare compared to other regions of the protein, but some of them reduce recognition by the neutralizing monoclonal antibody CR3022. This is consistent with the functional role of RBD in interacting with ACE2 and the assumption that new variants of the virus arise due to pressure from the host's immune system [24]. Virus strains with the 614Gly variant show significantly increased replication in human lung epithelial cells *in vitro* and increased titers in nasal and tracheal lavages of patients. Thus, the 614Gly variant increases the virus adaptability to persistence in the upper respiratory tract [25].

The SARS-CoV-2 genome encodes an RNA-dependent RNA polymerase (RdRp; also called Nsp12), which is involved in viral replication. Each of the two amino acid substitutions (Phe479Leu and Val556Leu) in the gene encoding RdRp confers significant resistance to remdesivir, an analog of adenosine [26, 27].

Untranslated flanking regions (5'- and 3'-untranslated region, 5'- and 3'-UTR) of the SARS-CoV-2 genome encode exclusively conserved secondary RNA structures with gene-regulating functions in viral replication and transcription. UTRs can interact with a number of human and viral protein factors and provide RNA – RNA or RNA – protein interactions due to genome circulation. To investigate the genomic stability of SARS-CoV-2, nucleotide variants in isolates collected during the ongoing pandemic were analyzed. 87 variants (SNPs) were identified with a frequency of higher than 0.5% (occurring in at least 93 genomes). In an extended analysis of 18,599 SARS-CoV-2 genomes, the g.241C>T variant was detected with a frequency of 70.2%. In addition, 6 variants were identified in 3'-UTR (g.29700A>G, g.29711G>T, g.29734G>C, g.29742G>T, g.29742G>A, g.29870C>A) and 3 variants in the 5'-UTR (g.36C>T, g.187A>G, g.241C>T), which were detected with a frequency of 0.62–1.05% [28]. A. Mishra et al. [29] identified two positions corresponding to the two substitutions revealed in this analysis, namely g.241C>T in the 5'-UTR and g.29742G>A/T in the 3'-UTR. If SNP occurs randomly, the probability that it results in a missense, synonymous, and nonsense mutation is 73%; 22%, and 5%, respectively, in all 26 viral genes encoding proteins. Analysis of noted amino acid substitutions in 769 SNPs with a variant frequency of 0.05% or higher revealed fewer than expected missense and nonsense mutations in all genes except *ORF8*. Deviations of the registered proportions from expected values varied widely across genes. In *ORF8*, for example, the frequency of missense, synonymous, and nonsense mu-

tations was 77%; 15%, and 8% respectively, which is close to expectations. In contrast, for the processed peptide nsp9 (non-structural protein 9), which putative function consists in dimerization and binding of RNA, the corresponding proportions were 18.2%; 81.8%, and 0%, respectively. Selection and evolution pressure probably differ in individual SARS-CoV-2 genes. Thus, the characterization of SARS-CoV-2 variants suggests a non-random selection pressure that points to hidden driving forces of evolution of the viral genome, associated with a functional or regulatory role [28].

Analysis of linkage disequilibrium (LD) of SNPs in 18,599 genomes identified a total of 34 groups of coevolving variants (CEV) with a frequency of ≥0.1%. The two groups of CEVs included UTR and other gene traits that may indicate functional dependencies or interactions of genomic elements carrying variants. The group 1 of CEV (CEVg1), detected in 69.5% of SARS-CoV-2 genomes, consisted of four variants located in the 5'-UTR (g.241C>T), nsp3 (g.3037C>T, synonym), the RNA-dependent RNA polymerase gene (g.14408C>T, p.P323L), and the S-protein gene (g.23403A>G, p.D614G). The incidence of CEVg1 increased sharply (from 12.2% to 93.4%) over the three-month period from February to May 2020 both globally and for each region by continents. The D614G mutation in CEVg1 increases the virus contagiousness. Another group of CEVs (CEVg5) associated with the 3'-UTR and found in 0.9% of genomes included 6 variants in the genes of the leader protein nsp1 (g.490T>A, p.D75E), nsp3 (g.3177C>T, p.P153L), exonuclease (g.18736T>C, p.F233L), S-protein (g.24034C>T, synonym), membrane protein (g.26729T>C, synonym) and the 3'-UTR itself (g.29700A>G). The CEVg5 group remained secondary in March-April 2020, amounting to 1.2% and 0.53%, respectively [28]. The nsp3 protein of coronaviruses is able to block the innate immune response of the host, and other non-structural proteins (nsp) play a role in evading recognition by the immune system [30]. In general, a review of variants in 18,599 SARS-CoV-2 genomes collected in May 2020 indicates that co-evolving and single variants with a probable functional effect on the replicative ability or pathogenicity of the virus have been identified both in the UTR and in functional elements throughout the genome [28].

More than 86,450 SARS-CoV-2 genomes became available in October 2020, therefore the group analysis of co-evolving variants increased the size of the first dataset of 18,599 genomes by more than 4 times. Comparison of the frequency of the CEV groups between the May and October 2020 datasets provided new insights into the evolution of SARS-CoV-2. First, it confirmed the global dominance of CEVg1 with the D614G mutation in the S-protein, which increased from 69.53% to 84.77% between May and October 2020. Second, the CEVg3 and CEVg4 groups gradually disappeared. Thirdly, two new groups of emerging co-evolving mutations (CEVg6 and CEVg8) were identified, which showed a rapid increase in frequency over a short period of time only on one continent and did not appear on other continents; for example, CEVg6 appeared in Oceania (its incidence increased from 0% in April to 96% in July 2020), while CEVg8 appeared in Europe (with an incidence of 0% in June and 36% in September 2020). The CEVg6 and CEVg8 groups carry new mutations in the S-protein, S477N and A222V, respectively [28].

Human microRNAs (miRNAs) represent evolutionarily conserved noncoding RNAs that can suppress gene expression posttranscriptionally due to hybridization of partially homologous sequences, primarily with 3'-UTR RNA. Human miRNAs can target viral RNAs and modulate positively or negatively various stages of viral replication and the viral life cycle [31]. To gain insight into the possible interaction of SARS-CoV UTRs with host microRNAs in modulation of the infection pathogenesis, we searched for sequence homology of human miRNAs with SARS-CoV-2 UTR sequences. A total of 8 microRNAs were identified from the miRBase database, including sense and antisense sequences corresponding to the 3'- and 5'-UTRs. Three miRNAs (hsa-miR-1307-3p, hsa-miR-1304-3p, and hsa-miR-15b-5p) are expressed in all 23 tissues, including lungs, heart, liver, kidneys, and small intestine, which are severely affected by SARS-CoV-2 infection. Sequences homologous to human hsa-miR-1307-3p and hsa-miR-1304-3p are located in S2m, a conserved genetic element of the virus with unknown function. Based on *in silico* computer modeling of the interaction between the viral 3'-UTR and human hsa-miR-1307-3p, a possible mechanism of viral survival has been presented, according to which a mutation in the 3'-UTR of SARS-CoV-2 weakens the host immune response. M. Khan et al. [32] identified a miR-1307-3p target in the 3'-UTR, which mediates antiviral responses and inhibits viral replication [33]. Previously, hsa-miR-1307-3p was associated with lung function [34], as well as with the progression of certain cancers in COVID-19 patients [35]. A study by L. Bavagnoli et al. demonstrated the functional role of human miR-1307 in the regulation of influenza A H1N1 virus replication [33] and predicted the complementarity of miR-1307 to the NS1 protein of the

https://doi.org/10.17816/clinpract64972

journal of clinical practice 2021 Vol 12 №1

H1N1 virus, which limits interferon and pro-inflammatory responses, allowing the virus to evade the innate and adaptive host immunity and replicate efficiently in infected cells. The C112A mutation, which allows the virus to evade miR-1307, is associated with acute respiratory distress syndrome. It is noteworthy that in the SARS-CoV-2 genome, the site of interruption of hybridization with miR-1307-3p coincides with the localization of the C112A mutation in the H1N1 genome. Apparently, SARS-CoV-2 shares a defense mechanism with H1N1 against host immunity, if SARS-CoV -2 carries an allele that weakens the function of miR-1307. In support of this hypothesis, analysis of SARS-CoV-2 variations revealed two nearby mutations at positions 29742 and 29734, which correspond to positions 7 and 15 of miR-1307, respectively. Mutations at these two sites can disrupt the hybridization of SARS-CoV-2 RNA with miR-1307 to avoid inhibiting infection. As of October 2020, these mutations were detected with a frequency of less than 1.2%. Their relationship with the severity of clinical symptoms is currently unknown and requires further study [28].

Thus, an integrated approach to the analysis of genome variations of circulating strains of SARS-CoV-2 during the current pandemic identified possible interactions of human miR-1307-3p microRNA with the 3'-UTR of the SARS-CoV-2 genome [28], which is confirmed by other researchers [36]. N. Balmeh et al. [36] identified hsa-miR-1307-3p as the best miRNA out of 1872 miRNAs with the highest affinity for the SARS-CoV-2 genome and associated cellular signaling pathways. The results of their study showed that this miRNA plays a regulatory role in the PI3K/Act signaling pathway, and is also involved in endocytosis and prevention of the production of the SARS-CoV-2 virus coreceptor, induced by hyperglycemia of the GRP78 (glucose regulating protein 78) protein, the expression of which increases in response on hyperklycemia in diabetes. Also, hsa-miR-1307-3p is involved in preventing the penetration and proliferation of the virus, which creates potential targets for antiviral interventions [36].

Currently, several variants of the Spike protein of the SARS-CoV-2 virus are known, which appeared as a result of mutations. It is unclear whether these variants may have a specific effect on the affinity for the ACE2 receptor, which in turn is characterized by multiple alleles in the human population. Among the 295,000 sequenced SARS-CoV-2 genomes isolated from different patients, several mutations in the Spike protein have been identified, that affect interactions with ACE2, namely S477N, N439K, N501Y, Y453F, E484K, K417N, S477I, and G476S. In particular, the N501Y mutation is one of the events characterizing the SARS-CoV-2 B.1.1.7 strain with increased infectivity, the frequency of which has recently increased in Europe [37].

A case of chronic infection with SARS-CoV-2 with reduced sensitivity to neutralizing antibodies in an immunosuppressed patient who received convalescent plasma treatment, which generates changes in the viral genome sequence, has been presented. The analysis covered 23 time points over 101 days. Minor changes were registered in the general structure of the viral population after two courses of remdesivir during the first 57 days. However, after plasma treatment, major dynamic changes in the virus population were found with the emergence of a dominant viral strain carrying mutations D796H in the S2 subunit and ΔH69/ΔV70 in the S1 subunit of the Spike protein. The D796H mutation turned out to be the main factor in reducing the virus sensitivity to plasma antibodies, but caused a defect in infectivity. Another mutation, the ΔH69/ΔV70 deletion, doubled the infectivity compared to the wild type and compensated for the decrease in infectivity resulting from the first D796H mutation. The Spike escape double mutant carrying the ΔH69/ΔV70 deletion and the D796H substitution had a moderately reduced sensitivity to antibodies in convalescent plasma *in vitro*, while retaining the infectivity similar to the wild type. These data indicate a strong selection of SARS-CoV-2 during convalescent plasma therapy, associated with the emergence of viral variants with reduced sensitivity to neutralizing antibodies [38].

The SARS-CoV-2 strain with a 382-nucleotide deletion (Δ382) in the *ORF8* gene appeared in Wuhan at the beginning of the pandemic. The Δ382 deletion truncates the open reading frame and interrupts transcription. The Δ382 variant causes a clinically significant disease, including pneumonia, but with a milder course compared to infections caused by wild-type virus. None (0%) of the 29 patients infected with this variant had hypoxia requiring supplemental oxygen (indicator of severe COVID-19, primary endpoint of the study), unlike patients infected with wild-type SARS-CoV-2 virus (28%). The clinical effect of deletions in the *ORF8* region is manifested by less systemic release of pro-inflammatory cytokines, less systemic inflammation, and a more effective immune response to SARS-CoV-2. Stronger production of IFN-γ at an early stage of infection, which was noted in patients infected with the Δ382 variant, supports the effector functions of T cells and a rapid and effective humoral response to SARS-CoV-2 [39].

The high transmissibility of the SARS-CoV-2 coronavirus by airborne and contact pathways has led to the COVID-19 pandemic, which continues to propagate throughout the world, despite strict control measures. Moreover, following the relaxation of social distancing policies, a resurgence of COVID-19 is registered in many regions. Whether real re-infection is possible is one of the key questions of COVID-19? Although neutralizing antibodies develop rapidly after infection, antibody titers start to decline as early as 1–2 months after acute infection. Patients with negative test result for SARS-CoV-2 RNA and those discharged from hospitals sometimes have positive retesting results. These reported cases have caused disagreement among experts about the hypothesis of persistent virus shedding and reinfection.

The study of the viral genome, in particular the sequencing of its sequence, is useful not only for tracking its variability and distribution, but also for clarifying the question of the possibility of re-infection. The first report of reinfection case was published in August 2020 in Hong Kong, when a 33-year-old man who recovered from COVID-19 in April and was discharged from hospital after two negative PCR tests for the presence of SARS-CoV-2 in swabs taken from nasopharynx and throat with an interval of 24 hours, had a positive test result for SARS-CoV-2 RNA in saliva after 4 months. During the second (asymptomatic) episode of COVID-19, the patient remained physically fit, and the blood test results were normal or nearly normal. Serial chest radiographs showed no abnormalities. The patient has not received antiviral treatment. The viral genomes from the episodes 1 and 2 belong to different strains of SARS-CoV-2. The viral genome 1 has a stop codon in the *ORF8* gene, leading to a truncation of 58 amino acids, and is phylogenetically associated with strains collected in March/April 2020, while the genome of the virus 2 is associated with strains collected in July/August 2020. Another 23 nucleotide and 13 amino acid differences located in 9 different proteins were revealed between the viruses from the episodes 1 and 2. Epidemiologic, clinical, serologic, and genomic analyzes confirmed that the patient had re-infection and not persistence of the virus after the first infection. These data indicate that SARS-CoV-2 may continue to circulate in humans despite herd immunity resulting from natural infection or vaccination [40]. Later, the possibility of re-infection was confirmed by other reports.

Thus, a 25-year-old man who lived in the USA was infected with SARS-CoV-2 twice, in April and June 2020. The second infection was symptomatically more severe than the first one. The genetic mismatch of SARS-CoV-2 samples in the two episodes of infection was greater than can be explained by the short-term *in vivo* evolution in the patient's body. These data indicate that the patient was infected with SARS-CoV-2 on two different occasions with genetically different strains of the virus. Thus, the previous exposure to SARS-CoV-2 does not guarantee the emergence of immunity against its new strains [41].

A report from Brazil described a series (33 cases) of reinfections, with 30 cases among healthcare workers. Sequencing of the viral genome revealed re-infection with a phylogenetically different isolate in each of these patients. Reinfection was associated with a decreased humoral response during the episode 1 of the disease and proves the need for constant vigilance without the assumption of the development of immunity in convalescents [42].

All authors of reports of cases of reinfection insist that patients who have recovered from COVID-19 must comply with epidemiological control measures.

Coronaviruses acquire genetic changes more slowly than other RNA viruses, due to correcting RNA-dependent RNA polymerase (RdRp). Repeated deletions in the S-protein gene that alter amino acid regions can stimulate and, apparently, accelerate the adaptive evolution of SARS-CoV-2. Deletion variants arise from different genetic and geographic backgrounds, are efficiently transmitted, and are present in new strains, including those that are causing the current global problem. Regions of the genome with recurrent deletion regions (RDR) are mapped with specific antibody epitopes. Deletions in the RDR confer resistance to neutralizing antibodies. For example, repeated deletions that change amino acids at positions 144/145 and 243-244 of the S protein disrupt the binding of the 4A8 antibody, which defines an immunodominant epitope in the N-terminal domain (NTD) of the S protein. Antigenic renewal of the virus allows re-infection of previously immunized individuals. During long-term infections in immunocompromised patients, the virus acquires deletions in the S-protein NTD. This process is called the "evolutionary pattern", defined by deletions that change certain epitopes. Deletions and substitutions in the major NTD and RBD epitopes will probably continue to contribute to this process. Unlike nucleotide substitutions, deletions cannot be corrected by RdRp polymerase correction, and this accelerates the adaptive evolution of SARS-CoV-2. Thus, deletions represent the mechanism by which rapid genetic and antigenic renewal of the S-protein of the SARS-CoV-2 virus occurs. Since

https://doi.org/10.17816/clinpract64972

journal of clinical practice 2021 Vol 12 №1

deletions represent a product of replication, they will occur at a certain rate, and these variants will probably appear in healthy populations [43].

HLA class I antigens play a crucial role in the development of a specific immune response to viral infections. M. Shkurnikov et al. [44] developed a risk scale associated with the ability of HLA class I molecules to represent peptides of the SARS-CoV-2 coronavirus. The scores on this scale are significantly higher in the group of adult patients who died from COVID-19 compared to old patients (p = 0.003). In particular, the presence of the HLA-A*01:01 allele is associated with a high risk of lethal outcome, while HLA-A*02:01 and HLA-A*03:01 are associated with a low risk. Analysis of homozygous patients showed that homozygosity for the HLA-A*01:01 allele is associated with early death of COVID-19 patients. Risk scores in an independent cohort of Spanish patients were also associated with disease severity. The results obtained indicate the important role of the presentation of viral peptides by HLA class I molecules in the development of a specific immune response to COVID-19. This conclusion is consistent with the data of Italian researchers that the occurrence of the HLA-A*01:01 and HLA-A*02:01 alleles is associated with the mortality rate in different regions of Italy [45]. To identify possible associations with clinical information, it is necessary to analyze the entire HLA class I genotype.

CONCLUSION

In this review, we aimed to highlight the available information on the genetic determinants of susceptibility to SARS-CoV-2 infection and the severity of COVID-19. The development of new drugs to treat this disease requires knowledge of its molecular pathways and critical target molecules. Blocking viral penetration pathways, including receptors and enzymes, and controlling immune responses are promising strategies for reducing multiple organ dysfunction.

ADDITIONAL INFORMATION

Author contribution. The authors made a substantial contribution to the conception of the work, acquisition, analysis, interpretation of data for the work, drafting and revising the work, final approval of the version to be published and agree to be accountable for all aspects of the work.

Funding source. Th is study was not supported by any external sources of funding.

Competing interests. The authors declare that they have no competing interests.

REFERENCES

1. WHO Coronavirus Disease (COVID-19) Dashboard [Internet]. Avalable from: https://covid19.who.int/
2. Ahmadian E, Khatibi SM, Soofiyani SR, et al. COVID-19 and kidney injury: pathophysiology and molecular mechanisms. *Rev Med Virol*. 2020;e2176. doi: 10.1002/rmv.2176
3. Sakurai A, Sasaki T, Kato S, et al. Natural history of asymptomatic SARS-CoV-2 infection. *N Engl J Med*. 2020;383(3):885–886. doi: 10.1056/NEJMc2013020
4. Clohisey S, Baillie JK. Host susceptibility to severe influenza A virus infection. *Crit Care*. 2019;23(1):303. doi: 10.1186/s13054-019-2566-7
5. Zhang Q, Bastard P, Liu Z, et al. Inborn errors of type I IFN immunity in patients with life-threatening COVID-19. *Science*. 2020;370(6515):eabd4570. doi: 10.1126/science.abd4570
6. Kaser A. Genetic risk of severe Covid-19. *N Engl J Med*. 2020;383(16):1590–1591. doi: 10.1056/NEJMe2025501
7. Ellinghaus D, Degenhardt F, Bujanda L, et al; Severe COVID-19 GWAS Group. Genomewide association study of severe COVID-19 with respiratory failure. *N Engl J Med*. 2020;383(16):1522–1534. doi: 10.1056/NEJMoa2020283
8. Pairo-Castineira E, Clohisey S, Klaric L, et al. Genetic mechanisms of critical illness in Covid-19. *Nature*. 2021;591(7848):92–98. doi: 10.1038/s41586-020-03065-y
9. Zhou S, Butler-Laporte G, Nakanishi T, et al. A Neanderthal OAS1 isoform protects against COVID-19 susceptibility and severity: results from mendelian randomization and case-control studies. *medRxiv*. 2020. doi: 10.1101/2020.10.13.20212092
10. Zeberg H, Pääbo S. The major genetic risk factor for severe COVID-19 is inherited from Neanderthals. *Nature*. 2020b;587(7835):610–612. doi: 10.1038/s41586-020-2818-3
11. Zeberg H, Pääbo S. A genomic region associated with protection against severe COVID-19 is inherited from Neanderthals. *Proc Natl Acad Sci USA*. 2021;118(9):e2026309118. doi: 10.1073/pnas.2026309118
12. WHO Solidarity Trial Consortium; Pan H, Peto R, Henao-Restrepo AM, et al. Repurposed antiviral drugs for Covid-19 – Interim WHO Solidarity Trial Results. *N Engl J Med*. 2021;384(6):497–511. doi: 10.1056/NEJMoa2023184
13. Varga Z, Flammer AJ, Steiger P, et al. Endothelial cell infection and endotheliitis in COVID-19. *Lancet*. 2020;395(10234):1417–1418. doi: 10.1016/S0140-6736(20)30937-5
14. Zhang C, Shi L, Wang FS. Liver injury in COVID-19: management and challenges. *Lancet Gastroenterol Hepatol*. 2020a;5(5):428–430. doi: 10.1016/S2468-1253(20)30057-1
15. Zhou Z, Ren L, Zhang L, et al. Heightened innate immune responses in the respiratory tract of COVID-19 patients. *Cell Host Microbe*. 2020;27(6):883–890.e2. doi: 10.1016/j.chom.2020.04.017
16. Hamming I, Timens WM, Bulthuis LC, et al. Tissue distribution of ACE2 protein, the functional receptor for SARS coronavirus. A first step in understanding SARS pathogenesis. *J Pathol*. 2004;203(2):631–637. doi: 10.1002/path.1570
17. Jia H, Thelwell C, Dilger P, et al. Endothelial cell functions impaired by interferon in vitro: Insights into the molecular mechanism of thrombotic microangiopathy associated with interferon therapy. *Thromb Res*. 2018;163:105–116. doi: 10.1016/j.thromres.2018.01.039
18. Stebbing J, Sánchez Nievas G, Falcone M, et al. JAK inhibition reduces SARS-CoV-2 liver infectivity and modulates inflammatory responses to reduce morbidity and mortality. *Sci Adv*. 2021;7(1):eabe4724. doi: 10.1126/sciadv.abe4724
19. Kuo CL, Pilling LC, Atkins JL, et al. APOE e4 Genotype Predicts Severe COVID-19 in the UK Biobank Community Cohort. *J Gerontol A Biol Sci Med Sci*. 2020;75(11):2231–2232. doi: 10.1093/gerona/glaa131
20. Gemmati D, Bramanti B, Serino ML, et al. COVID-19 and individual genetic susceptibility/receptivity: role of ACE1/ACE2 genes, immunity, inflammation and coagulation. Might the double X-chromosome in females be protective against SARS-CoV-2 compared to

the single X-chromosome in males? *Int J Mol Sci*. 2020;21(10):3474. doi: 10.3390/ijms21103474

21. Li Y, Zhang Z, Yang L, et al. The MERS-CoV Receptor DPP4 as a Candidate Binding Target of the SARS-CoV-2 Spike. *Science*. 2020b;23(8):101400. doi: 10.1016/j.isci.2020.101400

22. Lim S, Bae JH, Kwon HS, Nauck MA. COVID-19 and diabetes mellitus: from pathophysiology to clinical management. *Nat Rev Endocrinol*. 2021;17(1):11–30. doi: 10.1038/s41574-020-00435-4

23. Zeberg H, Pääbo S. The MERS-CoV receptor gene is among COVID-19 risk factors inherited from Neandertals. *bioRxiv*. 2020c. doi: 10.1101/2020.12.11.422139

24. Long SW, Olsen RJ, Christensen PA, et al. Molecular architecture of early dissemination and massive second wave of the SARS-CoV-2 virus in a major metropolitan area. *mBio*. 2020;11(6):e02707-20. doi: 10.1128/mBio.02707-20

25. Plante JA, Liu Y, Liu J, et al. Spike mutation D614G alters SARS-CoV-2 fitness and neutralization susceptibility. *bioRxiv*. 2020;2020.09.01.278689. doi: 10.1101/2020.09.01.278689

26. Shannon A, Le NT, Selisko B, et al. Remdesivir and SARS-CoV-2: structural requirements at both nsp12 RdRp and nsp14 exonuclease active-sites. *Antiviral Res*. 2020;178:104793. doi: 10.1016/j.antiviral.2020.104793

27. Gordon CJ, Tchesnokov EP, Woolner E, et al. Remdesivir is a direct-acting antiviral that inhibits RNAdependent RNA polymerase from severe acute respiratory syndrome coronavirus 2 with high potency. *J Biol Chem*. 2020;295(20):6785–6797. doi: 10.1074/jbc.RA120.013679

28. Chan AP, Choi Y, Schork NJ. Conserved genomic terminals of SARS-CoV-2 as coevolving functional elements and potential therapeutic targets. *mSphere*. 2020;5(6):e00754-20. doi: 10.1128/mSphere.00754-20

29. Mishra A, Pandey AK, Gupta P, et al. Mutation landscape of SARS-CoV-2 reveals three mutually exclusive clusters of leading and trailing single nucleotide substitutions. *bioRxiv*. 2020. doi: 10.1101/2020.05.07.082768

30. Meini S, Zanichelli A, Sbrojavacca R, et al. Understanding the pathophysiology of COVID-19: could the contact system Be the key? *Front Immunol*. 2020;11:2014. doi: 10.3389/fimmu.2020.02014

31. Girardi E, López P, Pfeffer S. On the importance of host microRNAs during viral infection. *Front Genet*. 2018;9:439. doi: 10.3389/fgene.2018.00439

32. Khan MA, Sany MR, Islam MS, Islam A. Epigenetic regulator miRNA pattern differences among SARS-CoV, SARS-CoV-2, and SARS-CoV-2 world-wide isolates delineated the mystery behind the epic pathogenicity and distinct clinical characteristics of pandemic COVID-19. *Front Genet*. 2020;11:765. doi: 10.3389/fgene.2020.00765

33. Bavagnoli L, Campanini G, Forte M, et al. Identification of a novel antiviral micro-RNA targeting the NS1 protein of the H1N1 pandemic human influenza virus and a corresponding viral escape mutation. *Antiviral Res*. 2019;171:104593. doi: 10.1016/j.antiviral.2019.104593

34. Herrera-Rivero M, Zhang R, Heilmann-Heimbach S, et al. Circulating microRNAs are associated with pulmonary hypertension and development of chronic lung disease in congenital diaphragmatic hernia. *Sci Rep*. 2018;8(1):10735. doi: 10.1038/s41598-018-29153-8

35. Qiu X, Dou Y. miR-1307 promotes the proliferation of prostate cancer by targeting FOXO3A. *Biomed Pharmacother*. 2017;88:430–435. doi: 10.1016/j.biopha.2016.11.120

36. Balmeh N, Mahmoudi S, Mohammadi N, Karabedianhajiabadi A. Predicted therapeutic targets for COVID-19 disease by inhibiting SARS-CoV-2 and its related receptors. *Inform Med Unlocked*. 2020;20:100407. doi: 10.1016/j.imu.2020.100407

37. Ortuso F, Mercatelli D, Guzzi PH, Giorgi F. Structural genetics of circulating variants affecting the SARS-CoV-2 spike/human ACE2 complex. *J Biomol Struct Dyn*. 2021;1–11. doi: 10.1080/07391102.2021.1886175

38. Kemp SA, Collier DA, Datir RP, et al. SARS-CoV-2 evolution during treatment of chronic infection. *Nature*. 2021. doi: 10.1038/s41586-021-03291-y

39. Young BE, Fong SW, Chan YH, et al. Effects of a major deletion in the SARS-CoV-2 genome on the severity of infection and the inflammatory response: an observational cohort study. *Lancet*. 2020;396(10251):603–611. doi: 10.1016/S0140-6736(20)31757-8

40. To KK, Hung IF, Ip JD, et al. COVID-19 re-infection by a phylogenetically distinct SARS-coronavirus-2 strain confirmed by whole genome sequencing. *Clin Infect Dis*. 2020:ciaa1275. doi: 10.1093/cid/ciaa1275

41. Tillett RL, Sevinsky JR, Hartley PD, et al. Genomic evidence for reinfection with SARS-CoV-2: a case study. *Lancet Infect Dis*. 2021;21(1):52–58. doi: 10.1016/S1473-3099(20)30764-7

42. Dos Santos LA, de Góis Filho PG, Fantini Silva AM, et al. Recurrent COVID-19 including evidence of reinfection and enhanced severity in thirty Brazilian healthcare workers. *J Infect*. 2021;82(3):399–406. doi: 10.1016/j.jinf.2021.01.020

43. McCarthy KR, Rennick LJ, Nambulli S, et al. Recurrent deletions in the SARS-CoV-2 spike glycoprotein drive antibody escape. *Science*. 2021;371(6534):1139–1142. doi: 10.1126/science.abf6950

44. Shkurnikov M, Nersisyan S, Jankevic T, et al. Association of HLA class I genotypes with severity of Coronavirus Disease-19. *Front Immunol*. 2021;12:641900. doi: 10.3389/fimmu.2021.641900

45. Pisanti S, Deelen J, Gallina AM, et al. Correlation of the two most frequent HLA haplotypes in the Italian population to the differential regional incidence of Covid-19. *J Transl Med*. 2020;18(1):352. doi: 10.1186/s12967-020-02515-5

AUTHORS INFO

The author responsible for the correspondence:
Aleksandr S. Golota, Cand. Sci. (Med.), Associate Professor; address: 9B Borisova st., 197706, Saint Petersburg, Sestroretsk, Russia; e-mail: golotaa@yahoo.com; eLibrary SPIN: 7234-7870; ORCID: https://orcid.org/0000-0002-5632-3963

Co-authors:
Dmitry A. Vologzhanin, Dr. Sci. (Med.); e-mail: volog@bk.ru; eLibrary SPIN: 7922-7302; ORCID: https//orcid.org/0000-0002-1176-794X

Tatyana A. Kamilova, Cand. Sci. (Biol.); e-mail: kamilovaspb@mail.ru; eLibrary SPIN: 2922-4404; ORCID: http://orcid.org/0000-0001-6360-132X

Olga V. Shneider, Cand. Sci. (Med.); e-mail: o.shneider@gb40.ru; eLibrary SPIN: 8405-1051; ORCID: https//orcid.org/0000-0001-8341-2454

Sergey G. Scherbak, Dr. Sci. (Med.), Professor; e-mail: b40@zdrav.spb.ru; eLibrary SPIN: 1537-9822; ORCID: https//orcid.org/0000-0001-5047-2792

https://doi.org/10.17816/clinpract64972