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SEQUENCING AND PHYLOGENETIC ANALYSIS OF THE ORF3A GENE VARIANT B.1.1.7 *SARS-COV-2* VIRUS

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Abstract. The problem of «new» infectious diseases has now acquired particular relevance for national and international health systems. One of these infections is the *SARS-CoV-2* coronavirus. Due to the rapid spread of a new type of coronavirus in the world, molecular genetic studies of this virus are of great importance. This will help to understand the nature of the virus and develop antiviral drugs to prevent the disease. The aim is to identify a missense mutation in the ORF3 gene of the studied coronavirus strain *SARS-CoV-2/human/KAZ/Britain*. This paper presents the results of the development and sequencing of the ORF3 gene of the coronavirus strain *SARS-Cov-2/human/KAZ/Britain*. For the development and sequencing by the Sanger method of the ORF3 gene, 4 pairs of primers were developed, with an overlap of 100-150 bp. The changes in the ORF3 gene compared to other strains whose data were obtained from the GISAID database were analyzed. As a result, the obtained amino acid sequences of the ORF3a gene of variant B.1.1.7 were compared with the reference strain HCoV-19/Wuhan/Hu-1/2019, where one mutation was determined at position 149: W (tryptophan) → L (leucine). The phylogenetic affiliation of the studied virus strain was also determined, where the genetic distance between the studied strains of the *SARS-CoV-2* virus was determined. Thus, the greatest similarity with the studied strain were:
HCoV-19/Wuhan/Hu-1/2019,
HCoV-19/England/MILK-9E05B3/2020,
HCoV-19/Japan/IC-0446/2020,
HCoV-19/Germany/NI-IOV-MHH15/2020,
and the strain *HCoV-19/Argentina/PAIS-F0418/2021* significantly distanced itself from the *SARS-CoV-2/human/KAZ/Britain strain*.
Keywords: *SARS-CoV-2*; mutation; PCR; primer; sequencing

Introduction

At the end of 2019, the republican office of the World Health Organization (WHO) in the People's Republic of China (PRC) was informed by the health authorities of the city of Wuhan about the registration of cases of pneumonia of unknown etiology [1, 2]. At the beginning of January 2020, 44 patients with pneumonia of unknown etiology were registered in China. The causative agent of the infection was not detected, and therefore WHO requested additional information from the national authorities of China to assess the risk [3]. Epidemiological studies conducted by the Chinese Center for Disease Control and Prevention (CDC China) and the Wuhan City Health Authority showed that the outbreak was associated with the seafood market, and with the help of genome-wide sequencing, a new (2019-nCoV) type of

coronavirus was identified, whose genome was about 29.9 bp. (GenBank NC_045512.2) [4, 5]. At the 5' end of the genome, 2019-nCoV encodes a replicase gene containing two open reading frames (ORF) ORF1 and ORF1b. The ORF gene contains 15 nonstructural proteins (NSP), including from NSP 1 to NCP10 and from NSP 12 to NSP 16 [5, 6]. At the 3' end, the genome contains the genes S, ORF3a, E, membrane protein (M), ORF6, ORF7a, ORF7b, ORF8, N and protein ORF10 [7, 8]. Among the proteins listed above, ORF3a, ORF8 and ORF10 are unique for a new coronavirus infection [9].

In this work, attention is focused on the ORF3a protein, since it participates in important aspects of the pathogenicity of the virus [10] and includes various functional domains depending on virulence, infectivity [11]. The ORF3a protein is located between the loci of the S and E genes, which forms the N-terminal transmembrane and C-terminal domains. The ORF3a - protein is viroporin, a viral transmembrane protein that exhibits the properties of ion channels in cell membranes [12, 13]. The ORF3a protein interacts with caveolin, which regulates various phases of the viral cycle [14]. It should be noted that a significant reaction of CD4+ and CD8+T cells to *SARS-CoV-2* in infected people was directed against ORF3a [15].

The ORF3a protein was initially found in the coronavirus family, and then described under various names, such as protein X1 [16], protein 3a [17], U274 [18] and ORF3a [14]. At the beginning of the COVID-19 pandemic in 2019, we learned about the pathogenic property of the ORF3a gene of the *SARS-CoV-2* virus [19]. Some studies have shown that many recovering patients with atypical pneumonia produce antibodies to ORF3a and the serum of patients with COVID-19 also demonstrates a high level of IgG and IgA reactivity to ORF3a, in addition to structural proteins [20]. The analysis of nucleotide and protein sequences of infection of a new variant of the coronavirus *SARS-CoV-2* provides important data on the biology of the virus. Therefore, molecular genetic studies are important for monitoring the evolution of the virus.

The aim of this work is to identify changes in the ORF3a gene of the studied coronavirus strain *SARS-CoV-2/human/KAZ/Britain*.

Materials and methods

Research material. The coronavirus strain *SARS-CoV-2/human/KAZ/Britain* from the laboratory of the collection of microorganisms of the Research Institute of Biological Safety Problems was used as the object of the study.

Isolation of RNA. Virus RNA was extracted from a virus-containing liquid with the QIAamp Viral RNA Mini kit (Qiagen, Germany) in accordance with the manufacturer's instructions in the BSL-3 laboratory.

cDNA synthesis. Reverse transcription (OT) was performed using the SuperScript VILO cDNA Synthesis Kit (Invitrogen, Thermo Fisher Scientific, USA) in a Mastercycler X50s thermal cycler at 25°C for 10 min; 42°C for 60 min; 85°C for 5 min.

Selection and synthesis of primers. The search and selection of specific primers for the development and sequencing of the ORF3a gene of the *SARS-CoV-2* virus was carried out using the online program Primer-BLAST (http://www.ncbi.nlm.nih.gov/tools/primer-blast). The synthesis of oligonucleotides was carried out on an automatic DNA/RNA Synthesizer H16 oligonucleotide synthesizer (K&A Laborgeraete, Germany) by the amidophosphite method.

Setting up PCR. Amplification was performed on a Mastercycler X50s thermal cycler using a Platinum Super Fi PCR Master Mix kit (Invitrogen, Thermo Fisher Scientific, USA) in accordance with the manufacturer's instructions. The operating time of PCR products was carried out according to the following temperature regime: initial denaturation 95°C-0.5 min; followed by 35 cycles of amplification at denaturation 95°C-0.1 min, annealing 57°C-0.5 min, elongation 72°C-0.5 min; final elongation 72°C-5 min.

Gel electrophoresis. Horizontal gel electrophoresis was performed in 1.5% agarose gel (Sigma, USA), stained with ethidium bromide, in a tris-acetate buffer at a voltage of 100 volts / cm of gel length, for 30 minutes with further detection on the MiniBIS Pro transilluminator (DNR Bio Imaging Systems, Israel). Visualization and documentation of the results of gel electrophoresis was carried out using the «GelCapture» program 1 kb DNA Ladder ready-to-use (Bioron, Germany) was used as a marker of molecular weights.

Purification of the PCR product. The PCR purification of the product was carried out with the GeneJET PCR Purification Kit (Thermo Fisher Scientific, USA) according to the manufacturer's instructions.

Sequencing by the Sanger method. Sequencing of the ORF3a gene of the *SARS-CoV-2* virus after purification of the PCR product was carried out using cyclic sequencing by the Sanger method with a set of AB BigDye Terminator v3.1 Cycle Sequencing Kit (Thermo Fisher Scientific, USA) and sequencing primers (Table 1), which were used at the production stage. The products were purified with a set of BigDye Xterminator (Thermo Fisher Scientific, USA) and sequenced using a 3130 XL genetic analyzer (Applied Biosystems).

Bioinformatic analysis and algorithm. After sequencing, the obtained nucleotide sequence data were processed using the Sequencher v.5.4 program (Gene Codes Corporation, USA). Comparison of the amino acid sequence of the ORF3 gene with the reference strain was carried out using the Protein BLAST program (https://blast.ncbi.nlm.nih.gov) based on NCBI. Multiple alignment, phylogenetic analysis and calculation of genetic distances of the gene were carried out using the MEGA 11 program [21, 22].

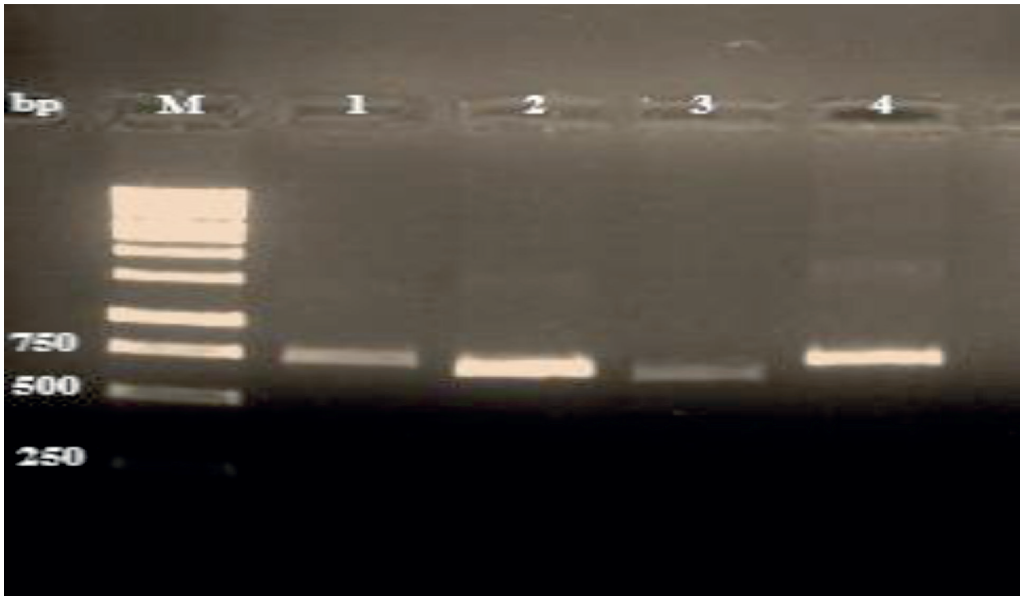
The main results of the research. The sequence of nucleotides of specific primers was selected based on the ORF3 gene of the reference strain *Wuhan-Hu-1*. Primers were selected in such a way that each pair of primers overlapped each other. As a result, 4 pairs of sequencing primers were selected (Table.1) to develop the complete ORF3a gene of the *SARS-CoV-2* virus with an overlap of about 100 bp.

Table 1 – Parameters of sequencing primers

№	Sequence 5' – 3'	Length	Tm	GC%	Position	Product size, bp
f	Forward primer TGACTTATGTCCCTGCACAA	20	56.76	45.00	24756-24775	731
r	Reverse primer GTAGCGCGAACAAAATCTGA	20	56.85	45.00	25487-25468	
f	Forward primer TGTGTCTGGTAACTGTGATGT	21	56.88	42.86	24925-24945	686
r	Reverse primer GAGTGCTAGTTGCCATCTCT	20	57.02	50.00	25611-25592	
f	Forward primer CGATACCGATACAAGCCTCA	20	56.92	50.00	25493-25512	654
r	Reverse primer GAACCGTCGATTGTGTGAAT	20	56.47	45.00	26147-26128	
f	Forward primer GCCGTTCCAAAAACCCATTA	20	56.89	45.00	25790-25809	743
r	Reverse primer GAACCTGCCATGGCTAAAAT	20	56.35	45.00	26533-26514	

Selected primers (Table.1) allow to fully develop the ORF3a gene of the *SARS-CoV-2* virus, the length of which is 825 bp [19].

Thus, with the selected primers (Table 1), 4 fragments of the ORF3a gene of the *SARS-CoV-2* virus were developed (Fig.1).



M – marker; 1–4 test samples

Figure 1 – Electrophoregram of PCR products of the *ORF3a* gene strain *SARS-CoV-2/human/KAZ/Britain*.

As can be seen from Figure 1, after carrying out standard PCR with a set of specific primers (Table 1), fragments of PCR products were developed in appropriate sizes.

As a result, the amino acid sequences of the obtained ORF3a gene were compared with the reference strain, where one mutation was detected at position 149 (Fig.2).

Download Graphics

unnamed protein product

Sequence ID: Query_16105 Length: 275 Number of Matches: 1

Range 1: 1 to 275 Graphics ▼ Next Match ▲ Previous Match

Score	Expect	Method	Identities	Positives	Gaps
565 bits(1456)	0.0	Compositional matrix adjust.	274/275(99%)	274/275(99%)	0/275(0%)

```
Query  1    MDLFMRIFTIGTVTLKQGEIKDATPSDFVRATATIPIQASLPFGWLIVGVALLAVFQSAS  60
Sbjct  1    ............................................................  60

Query  61   KIITLKKRWQLALSKGVHFVCNLLLLFVTVYSHLLLVAAGLEAPFLYLYALVYFLQSINF  120
Sbjct  61   ............................................................  120

Query  121  VRIIMRLWLCWKCRSKNPLLYDANYFLCLHTNCYDYCIPYNSVTSSIVITSGDGTTSPIS  180
Sbjct  121  ............................W...............................  180

Query  181  EHDYQIGGYTEKWESGVKDCVVLHSYFTSDYYQLYSTQLSTDTGVEHVTFFIYNKIVDEP  240
Sbjct  181  ............................................................  240

Query  241  EEHVQIHTIDGSSGVVNPVMEPIYDEPTTTTSVPL  275
Sbjct  241  ...................................  275
```

Figure 2 – Comparison of the amino acid sequence of the analyzed ORF3a gene with the reference strain Wuhan-Hu-1.

As a result of the data obtained, a variation was determined that led to a corresponding change in the amino acid from W (Tryptophan) to L (Leucine) at the amino acid position 149.

Next, a phylogenetic analysis of the studied viral strain by the ORF3a gene was performed in order to determine the most phylogenetically close to it viral strains of SARS-CoV-2 (Fig.3). The analysis used 12 other global strains obtained from the database of the Global Initiative for the Exchange of All Influenza Data (GISAID) (*https://www.gisaid.org*).

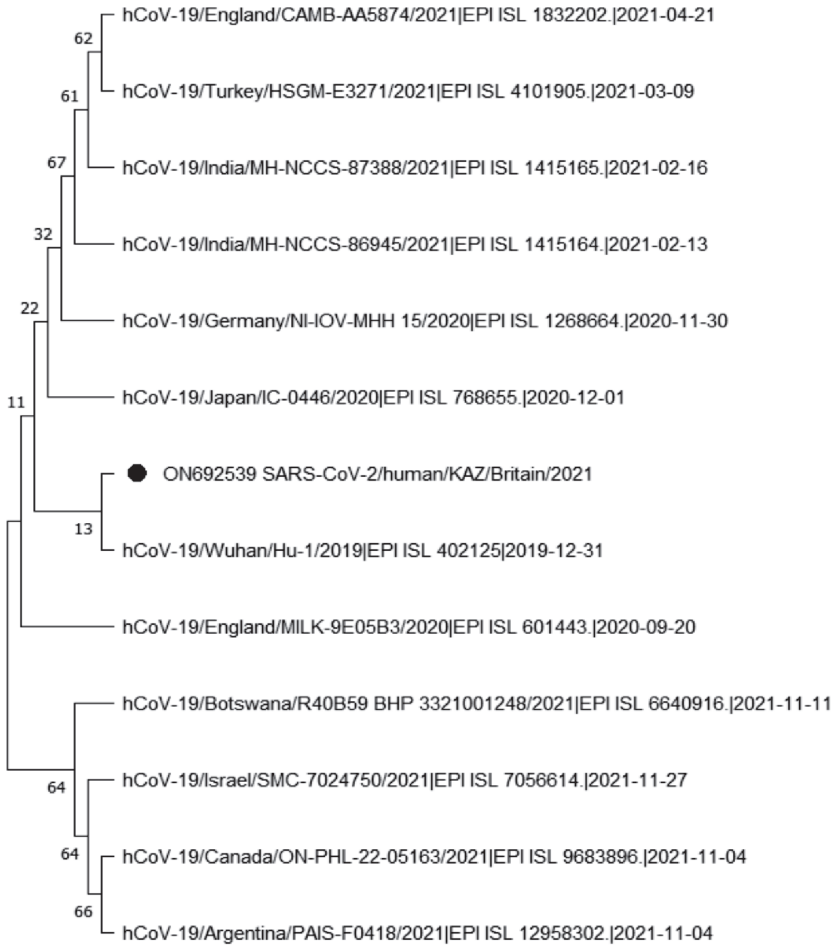


Figure 3 – Phylogenetic tree of the *SARS-CoV-2/human/KAZ/Britain* strain and other *SARS-CoV-2* strains by the ORF3a gene.

Phylogenetic analysis showed that the viral strains *SARS-CoV-2/human/KAZ/Britain* and *HCoV-19/Wuhan/Hu-1/2019* are identical to each other in this part of the genome.

When analyzing the assessment of the evolutionary divergence between the sequences of the ORF3 gene of the strain under study and the data (Fig.2) of *GISAID*, the number of basic differences between the nucleotide sequences of the gene was determined (Table 2).

Table 2 – Genetic differentiation of the ORF3a gene of the *SARS-CoV2/human/KAZ/Britain* strain and other strains obtained from the GISAID database

№	Name of strains	1	2	3	4	5	6	7	8	9	10	11	12	13
1	SARS-CoV-2/human/KAZ/ Britain		0,00120	0,00120	0,00120	0,00120	0,00124	0,00124	0,00162	0,00216	0,00178	0,00178	0,00178	0,00255
2	hCoV-19/Wuhan/Hu-1/2019	0,00121		0,00000	0,00000	0,00000	0,00000	0,00000	0,00115	0,00179	0,00130	0,00130	0,00130	0,00215
3	hCoV-19/England/ MILK-9E05B3/2020	0,00121	0,00000		0,00000	0,00000	0,00000	0,00000	0,00115	0,00179	0,00130	0,00130	0,00130	0,00215
4	hCoV-19/Japan/IC-0446/2020	0,00121	0,00000	0,00000		0,00000	0,00000	0,00000	0,00115	0,00179	0,00130	0,00130	0,00130	0,00215
5	hCoV-19/Germany/ NI-IOV-MHH15/2020	0,00121	0,00000	0,00000	0,00000		0,00000	0,00000	0,00115	0,00179	0,00130	0,00130	0,00130	0,00215
6	hCoV-19/India/ MH-NCCS86945/2021	0,00126	0,00000	0,00000	0,00000	0,00000		0,00000	0,00000	0,00132	0,00134	0,00134	0,00134	0,00223
7	hCoV-19/India/ MH-NCCS87388/2021	0,00126	0,00000	0,00000	0,00000	0,00000	0,00000		0,00000	0,00132	0,00134	0,00134	0,00134	0,00223
8	hCoV-19/England/ CAMB-AA5874/2021	0.00243	0,00121	0,00121	0,00121	0,00121	0,00000	0,00000		0,00127	0,00178	0,00178	0,00178	0,00244
9	hCoV-19/Turkey/ HSGM-E3271/2021	0.00365	0.00243	0.00243	0.00243	0.00243	0,00126	0,00126	0,00121		0,00221	0,00221	0,00221	0,00275
10	hCoV-19/ Botswana /R40659 BHP 3321001248 /2021	0.00243	0,00121	0,00121	0,00121	0,00121	0,00125	0,00125	0,00242	0,00364		0,00000	0,00000	0,00175
11	hCoV-19/Israel/ SMC-7024750/2021	0.00243	0,00121	0,00121	0,00121	0,00121	0,00125	0,00125	0,00242	0,00364	0,00000		0,00000	0,00175
12	hCoV-19/Canada/ ON-PHL-22-05163/2021	0.00243	0,00121	0,00121	0,00121	0,00121	0,00125	0,00125	0,00242	0,00364	0,00000	0,00000		0,00175
13	hCoV-19/Argentina/ PAIS-F0418/2021	0.00486	0,00364	0,00364	0,00364	0,00364	0,00377	0,00377	0,00485	0,00609	0,00242	0,00242	0,00242	

From the data obtained, it can be seen that the nucleotide sequence of the ORF3a gene showed the greatest similarity between the strains *SARS-CoV-2/human/KAZ/Britain* and *HCoV-19/Wuhan/Hu-1/2019, HCoV-19/England/MILK-9E05B3/2020, HCoV-19/Japan/IC-0446/2020, HCoV-19/Germany/NI-IOV-MHH15/2020,* the divergence coefficient of which is D≥0.00121. Pairwise values of genetic differentiation (Table 2) based on the ORF3a gene showed that the strain *SARS-CoV-2/human/KAZ/Britain* significantly distances itself from the strain *HCoV-19/Argentina/PAIS-F0418/2021* (D ≥ 0.00486).

Discussion of the results obtained. According to data from *GISAID*, there are currently 12 different clades of the *SARS-CoV-2* virus in the world (www.gisaid.org). A significant place for the evolutionary control and differentiation of mutational variability of microorganisms is occupied by molecular genetic studies. Sanger sequencing is a reference sequencing method in which the probability of incorrect reading is very low compared to other next-generation sequencing methods.

In this research paper, we presented data on the sequencing of the ORF3a gene of the *SARS-CoV-2* virus. For these purposes, we have developed 4 pairs of sequencing primers, which together develop the whole ORF3a gene of the *SARS-CoV-2* coronavirus (Fig.1). During the research, the optimal parameters of the PCR formulation were determined, which corresponds to the following values: the annealing temperature is 57°C, and the concentration of each primer is 400 mM in the reaction mixture. According to the WHO classification, mutations in the ORF3a gene were detected in the groups of virus of interest (VOI) and virus of concern (VOC) (Table 3) [19].

Table 3 – Brief description of new mutant variants for the *ORF3a* gene of the *SARS-CoV-2* virus [19]

Вирусные варианты	Pango lineage	First detection and location detection	ORF3a mutations
Virus of Interest (VOI)			
Lambda	C.37	Peru, December - 2020	No
Mu	B.1.621	Colombia, January - 2021	Q57H, del256/257
Epsilon	B.1.427	California, USA, June - 2020	Q57H
Virus of Concern (VOC)			
Alpha	B.1.1.7	United Kingdom, September - 2020	No
Beta	B.1.351	South Africa, May - 2020	Q57H, S171L
Gamma	P.1	Brazil, November - 2020	S253P, D155Y
Delta	B.1.617.2	India, October - 2020	S26L
Omicron	BA.1/B.1.1.529	South Africa November - 2021	No
	BA.2/B.1.1.529.2	South Africa November - 2021	T223I
	BA.3/B.1.1.529.3	South Africa November - 2021	T223I

As can be seen from Table 3, the Q57H mutation is present in the «Epsilon» and Q57H/S171L «Beta» variants [23]. The appearance of the Q57H mutation is associated with the 4th wave of the revival of SARS-CoV-2 in Hong Kong [24]. In the «Gamma» variant, only a single S253P change or combined S253P/D155Y mutations were detected. Among the sarbecovirus group, D155 and S253 residues are considered to be the most conservative regions, and these mutations cause a change in the activity of the ORF3a protein [19]. The S26L mutation found in the «Delta» variant does not affect the nature of the virus, since this region is very diverse among the sarbecovirus family [25]. Currently, there is no evidence that the T223I mutation found in the «Omicron» variant affects the virulence of the virus [26, 27].

In this work, we identified a change in one amino acid in the ORF3a gene of the *SARS-CoV-2/human/KAZ/Britain* strain. Davarynejad et.al [28] showed that replacing just one

amino acid in one protein is crucial for cell division. This requires regular genomic and epidemiological studies to identify new variants of viral pathogens. Since the amino acid sequence was detected, it was found only in the strain under study compared to other strains of *SARS-CoV-2* variants (https://cov-lineages.org/global_report_B.1.1.7.html). This identified amino acid sequence requires effective research in the future, since it is unknown whether this affects the virulence and functional nature of the virus.

Phylogenetic analysis showed that the studied strain is close to the reference strain compared to other variants of *SARS-CoV-2* strains.

However, social distancing of the population, non-compliance with personal protective measures and low vaccination rates also play a significant role in the spread of coronavirus infection. Thus, molecular genetic analysis further activates the need for genomic studies to identify new changes in the genes of *SARS-CoV-2* variants.

Conclusion. As a result of research work, four pairs of primers were selected for the development and sequencing by the Sanger method of the ORF3a gene of variant B.1.1.7, with a 100-150 bp overlap. The results of the study showed that PCR products fully correspond to the length of the developed primers.

The obtained amino acid sequences of the ORF3a gene were compared with a reference strain (GenBank inventory number NC_045512.2) where one mutation was determined at position 149: W→L. Comparative genetic analysis showed that the nucleotide sequences of the ORF3a gene of the coronavirus strain *SARS-CoV-2/human/KAZ/Britain* are highly identical to the ORF3a gene of the *HCoV-19/Wuhan/Hu-1/2019* strain.

The analysis of the nucleotide sequence of the ORF3a gene of the *SARS-CoV-2/human/KAZ/Britain* strain showed the greatest similarity with *HCoV-19/Wuhan/Hu-1/2019*, *HCoV-19/England/MILK-9E05B3/2020*, *HCoV-19/Japan/IC-0446/2020*, *HCoV-19/Germany/NI-IOV-MHH15/2020*. The genetic distances revealed by the nucleotide sequences of the *ORF3a* gene are significantly distanced from *HCoV-19/Argentina/PAIS-F0418/2021*.

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The authors of this article declare that there is no conflict of interest.

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СЕКВЕНИРОВАНИЕ И ФИЛОГЕНЕТИЧЕСКИЙ АНАЛИЗ ГЕНА ORF3A ВАРИАНТА B.1.1.7 ВИРУСА *SARS-COV-2*

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Аннотация: Проблема «новых» инфекционных болезней в настоящее время приобрела особую актуальность для национальных и международных систем здравоохранения. Одной из таких инфекций является коронавирус *SARS-CoV-2*. В связи с быстрым распространением нового типа коронавируса в мире, важное место занимает проведение молекулярно-генетических исследований данного вируса. Это поможет понять природу вируса и разработать противовирусные препараты с целью профилактики заболевания. Цель - идентификация миссенс-мутации в гене ORF3a исследуемого коронавирусного штамма *SARS-CoV-2/human/KAZ/Britain*. В данной работе представлены результаты наработки и секвенирования гена ORF3a коронавирусного штамма *SARS-CoV-2/human/KAZ/Britain*. Для наработки и секвенирования методом Сэнгера гена ORF3a было разработано 4 пары праймеров, с перектытием 100-150 п.о. Анализированы изменения в гене ORF3a по сравнению с другими штаммами, данные которых получены из базы данных GISAID. В результате полученные последовательности аминокислот гена ORF3a варианта B.1.1.7

были сравнены с эталонным штаммом *hCoV-19/Wuhan/Hu-1/2019*, где была определена одна мутация в позиции 149: *W* (триптофан) → *L* (лейцин). Также определена филогенетическая принадлежность исследуемого штамма вируса, где определена генетическая дистанция между исследованными штаммами вируса *SARS-CoV-2*. Таким образом, наибольшее сходство с исследуемым штаммом были:

hCoV-19/Wuhan/Hu-1/2019,
hCoV-19/England/MILK-9E05B3/2020,
hCoV-19/Japan/IC-0446/2020,
hCoV-19/Germany/NI-IOV-MHH15/2020,

а штамм *hCoV-19/Argentina/PAIS-F0418/2021* значительно дистанцировал от штамма *SARS-CoV-2/human/KAZ/Britain*.

Ключевые слова: *SARS-CoV-2*; мутация; ПЦР; праймер; секвенирование

SARS-COV-2 ВИРУСЫ B.1.1.7 ВАРИАНТЫНЫҢ ORF3A ГЕНІН СЕКВЕНИРЛЕУ ЖӘНЕ ФИЛОГЕНЕТИКАЛЫҚ ТАЛДАУ

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Аннотация: «Жаңа» жұқпалы аурулар проблемасы қазіргі уақытта ұлттық және халықаралық денсаулық сақтау саласы үшін ерекше өзектілікке ие. Осындай инфекциялардың бірі-жаңа *SARS-CoV-2* коронавирусы. Коронавирустың жаңа түрінің әлемде тез таралуына байланысты вирус биологиясына молекулалық - генетикалық зерттеулер жүргізу маңызды орын алады. Бұл вирустың табиғатын түсінуге және аурудың алдын алу үшін вирусқа қарсы препараттарды жасауға көмектеседі. Жұмыстың мақсаты - зерттелетін *SARS-CoV-2/human/KAZ/Britain* коронавирустық штамының ORF3a геніндегі миссенс-мутацияны анықтау. Бұл жұмыста *SARS-CoV-2/human/KAZ/Britain* коронавирус штамының ORF3a генінің амплификациясы мен секвенирлеу нәтижелері ұсынылған. ORF3a генін амплификация және Сэнгера әдісімен секвенирлеу үшін қабаттасуы 100 – 150 ж.н. құрайтын 4 жұп праймер жасалынды. GISAID деректер қорынан алынған басқа штамдармен салыстырғанда ORF3a геніндегі өзгерістер талданды. B.1.1.7 нұсқасы ORF3a генінің аминқышқылдарының тізбегін эталондық *hCoV-19/Wuhan/Hu-1/2019* штамымен салыстырылды, нәтижесінде 149 позициясында бір мутация анықталды: W (триптофан) → L (лейцин). Сондай-ақ зерттелетін вирус штамының филогенетикалық сәйкестілігі мен *SARS-CoV-2* вирустарының зерттелген штамдарының арасындағы генетикалық қашықтығы анықталды. Зерттелген штамға ең үлкен ұқсастық:

hCoV-19/Wuhan/Hu-1/2019,
hCoV-19/England/MILK-9E05B3/2020,
hCoV-19/Japan/IC-0446/2020,
hCoV-19/Germany/NI-IOV-MHH15/2020,

ал *hCoV-19/Argentina/PAIS-F0418/2021* штамы *SARS-CoV-2/human/KAZ/Britain* штамынан айтарлықтай алыстады.

Кілтті сөздер: *SARS-CoV-2*; мутация; ПЦР; праймер; секвенирования