Genetic instability of the Omicron strain of the SARS-CoV-2 virus.

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Abstract. Among the mutations found in the Omicron strain, the results of cytosine deamination dominate. There is a mutation in the nsp14 gene. These two facts suggest that the omicron strain has an impaired repair system. The instability of the genome of the Omicron strain to the action of APOBEC deaminases will most likely lead to the degradation of this strain. However, the same mutations have led to several dangerous properties of the Omicron strain. It is proposed to use the instability of the Omicron strain to deamination of cytosine for the prevention of a severe course of the disease.

Discovered in November 2021, the new Omicron strain of the SARS-CoV-2 virus has a high transmissibility and, according to preliminary estimates, is able to displace the currently circulating varieties of the Delta strain [1]. In this regard, it is useful to understand the potential and dangers of the existence of a new strain.

The Omicron strain resulted from the accumulation of more than 60 mutations, compared to the original Wuhan 2019 strain [2]. The large number of mutations and the absence of transitional forms between the Wuhan strain and the omicron suggests unusual scenarios of evolution for the Omicron strain. For example, the possibility of the appearance of this strain as a result of the persistence of the virus in the body of a patient with HIV is widely discussed [3]. Also, a large number of mutations may result from the loss of the ability of the virus to resist mutations. The possible genetic instability of the Omicron strain is interesting both from the point of view of the evolutionary prospects of this strain, and from the point of view of the prevention of therapy for COVID-19 caused directly by the Omicron strain.

An idea of the genetic instability of the Omicron strain can be obtained by analyzing the nature of mutations that have arisen in its genome. So, for example, seven out of ten synonymous mutations account for deamination of cytosine C → U [4]. Of the 45 mutations leading to amino acid substitutions, 23 are explained by deamination of cytosine in the coding sequence of the RNA of the Omicron strain. The nature of the mutations may indicate that the genome of the Omicron strain, for some reason, has become less resistant to cytosine deamination. In addition, the rate of accumulation of mutations in comparison with the Delta strain increased, which also requires explanation.

There is a single I42V mutation in the nsp14 gene of the Omicron strain. Currently, quite a lot is known about the key role of the nsp14 protein in the stability of the coronavirus genome [5-11]. The nsp14 protein is an important component of the genome repair system. This change may be the key to explaining the loss of resistance of the viral genome to deamination. nsp14 works in combination with polymerase and allows you to neutralize the action of APOBEC cytosine deaminases. 12 deamination of cytosine directly in the genome of the virus led to amino acid

substitutions. Another 13 deamination of cytosine occurred in the complementary strand, which led to the substitution of guanine for adenine directly in the RNA of the virus and amino acid substitutions. This is a huge number of replacements. Some of the amino acid substitutions are penalized by selection; therefore, the scale of the contribution of cytosine deamination to the appearance of the Omicron strain is better estimated by synonymous substitutions. Let me remind you that seven out of ten synonymous mutations account for cytosine deamination. All seven mutations occurred in the growing chain, that is, they were missed by the repair system based on the damaged nsp14.

Given the high likelihood that the Omicron strain has damaged nsp14 and genetic instability, it is necessary to analyze similar cases. Although similar phenomena have been studied in laboratories [10, 11], we are primarily interested in situations that are similar not at the molecular, but at the population level. A similar case was recently registered in Japan, a work on this topic was announced by Professor Ituro Inoue [12]. From an interview with Professor Inoue, it is known that the Delta strain accumulated several mutations in nsp14, was able to supplant the rest of the strains and disappeared due to a catastrophe of errors. Although an article or a preprint by Japanese geneticists on this topic has not yet been published, this statement is extremely important for further reasoning. If a strain with a broken repair system gains an advantage in spreading speed, then this must be explained. Perhaps saving on the repair system allows for faster production of copies of the virus, and this speed gain is decisive. The nsp14 protein is an exonuclease that cuts off up to 6 of the last nucleotides attached to the chain. That is, reparation can indeed be a very costly process. It would seem that a reverse mutation in the nsp14 gene could restore the repair system. This would just require a single cytosine deamination to replace V42I. Probably, such an event would significantly reduce the viral RNA copying rate and therefore is penalized by selection.

In the human body, viral RNA is exposed to the danger of deamination by enzymes of the APOBEC class. Protein nsp14 protected the genome of the SARS-CoV-2 virus from the action of these deaminases. Professor Inoue is a specialist in APOBEC enzymes [13], so one can trust his statement that these enzymes led to the disappearance of the Japanese variant of the Delta strain [12]. The Japanese case gives hope for the self-destruction of the Omicron strain. Also, it can be assumed that the drug from Merck Lagevrio (Molnupiravir) will be highly effective against this strain. The principle of action of this drug is based on the accelerated introduction of mutations that mimic the action of APOBEC deaminases [14]. Perhaps it was the similarity of the action of the drug from Merck and APOBEC that formed the basis for the assumption that Omicron arose as a result of clinical trials of this drug in South Africa [15]. I am inclined to believe that the most likely version is with long-term persistence of the virus in the body of an HIV-infected person. This version explains the possibility of long-term changes based on an ancestral strain that has long disappeared in South Africa. For additional support of this hypothesis, one can draw attention to the fact that an extremely high activity of APOBEC is sometimes observed in the body of HIV-infected people [16]. That is, not only the reduced ability to neutralize the action of APOBEC, but also the high activity of these deaminases in the patient's body could take part in the formation of the Omicron strain.

Currently, there are already positive expectations in the media related to the emergence and spread of the Omicron strain. Genetic instability can provide not only the expected degradation of the genome of the Omicron strain, but also an easier course of the disease. Since the drug

Molnupiravir is characterized by a milder course of the disease, it is natural to expect the same effect from the Omicron strain. Therefore, I would not like this text to be perceived as a confirmation of only positive expectations. It is already known that mutations in the S-protein allow the new strain to effectively escape from neutralization by antibodies of those who have been ill and vaccinated. The Omicron strain is capable of a disease requiring hospitalization even for young patients and children, which makes it similar to the Delta strain. Therefore, in the second part of the work, I would like to try to explain the existence of some dangerous sides in the Omicron strain. In this case, I will also rely on the analysis of mutations in the genome of the Omicron strain.

It would be hoped that cytosine deamination would lead to the disappearance of the GC-rich furin cleavage site. This did not happen, as the cleavage with furin provides a clear advantage in the infection of various types of human cells. Moreover, three mutations in the furin cleavage site of the Omicron strain suggest that the site is even more active [17]. As a result of two mutations, histidine moved within the furin cleavage site. As a result of the third mutation, asparagine was replaced by lysine. As a result, the positive charge of the furin site only increased, which ensures that the serine protease cleavage site is located on the surface of the S-protein.

More than 30 mutations occurred in the S-protein of the Omicron strain. There are 8 more positively charged amino acids in the S-protein structure. Could this negatively affect the properties of the S-protein? Unfortunately the answer is yes. An experimental study of the S-protein of the ancestral (Wuhan) strain showed that a decrease in pH promotes the transition of the S-protein to a less immunogenic conformation [18]. The review [19] analyzes the relationship between acidosis and the severe course of COVID-19 caused by the Wuhan strain. At the beginning of the pandemic, the risk group was represented exclusively by patients prone to metabolic acidosis. It is assumed that one of the mechanisms of the severe course of covid was a change in the conformation of the S-protein during the development of acidosis, which slowed down and hindered the production of antibodies. The appearance of an additional positive charge on the surface of the S-protein is equivalent to the protonation of surface groups in an acidic medium. The initially less immunogenic conformation of the surface protein for Omicron makes it possible to understand the expansion of the risk group. It should be noted that the delta strain was also able to accumulate a large amount of positively charged amino acids [20]. Of the seven amino acid substitutions, six increased the charge. It is noteworthy that in this case the contribution of cytosine deamination was completely absent. Nevertheless, the Delta strain is also characterized by the expansion of the boundaries of the risk group [21], which can also be associated with an increase in the stability of the less immunogenic conformation. It can be assumed that the result of fixing a less immunogenic conformation was evolutionarily achieved in two independent ways. In a sense, this result was predetermined by the need to avoid antibodies.

The Omicron strain contains qualities that make it potentially dangerous: high spreading rate, invisibility to antibodies of recovered and vaccinated patients, active furin cleavage site. Therefore, the question of methods of prevention and treatment of COVID-19 caused by the Omicron strain is relevant. Is it possible to use against this strain its Achilles heel, the instability to the action of APOBEC deaminases? All APOBEC deaminases are Zn-containing enzymes [22]. Prophylactic intake of Zn supplements can improve the prognosis of the course of the disease and reduce the risk of hospitalization for many viral infections [23]. COVID-19 is subject to general

rules [24]. It is likely that in the case of the Omicron strain, such additives will be especially effective. This forecast can be taken critically and made the object of serious scientific research. However, given the rate at which the Omicron strain is spreading, the simplest strategy is to start prophylactic Zn supplementation soon.

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