Studying the virulence of a rapidly mutating strain of seasonal influenza

Abstract

This study detected the vaccine-resistant population of A/Hong Kong/4801/2014 (H3N2) virus. A comparative analysis of virus genomes from two H3N2 carriers was performed. The virus population was mutated in one of the carriers at epitope C, which is one of epitope regions of the hemagglutinin H3 protein, responsible for binding to receptors on cells.

This study allows us to know the weaknesses of the existing vaccine and brings us closer to creating a new one.

Introduction

Seasonal influenza is a widespread relapsing respiratory infection in the middle lane countries that provokes prolonged epidemics each year. In the era of the coronavirus pandemic, it is easy to forget that influenza is a dangerous disease that kills up to half a million people each year [1]. Hospitals are overcrowded during the off-season, and flu epidemics place a heavy economic burden on the state. Although most patients recover the infection without much trouble, the long-term effects and complications may affect the least protected groups of the population: the elderly and people with chronic illnesses. Among them, the mortality rate is very high [2].

Since the flu virus mutates too quickly, lifelong immunity is not developed. Therefore, the only way to protect the population from serious consequences is to get an annual seasonal vaccination.The flu vaccine contains a set of antigens from several viral strains. Although advanced quadrivalent vaccines have been developed, traditional approaches to seasonal vaccination involve the use of a standard trivalent vaccine [3]. It contains 15 μg of the two A and B strains. In Particular, vaccines based on genetic fragments of A/H1N1, A/H3N2 and B influenza virus strains will be used in Russia from 2022-2023.

However, even vaccination does not provide complete protection against infection. The influenza virus genome mutates very quickly, leading to an increased probability a mutation will occur in the hemagglutinin (HA). This phenomenon is called antigenic drift [4].

We often forget about such an important factor as sequencing error. When it comes to the influenza virus, researchers isolate RNA from a multitude of virions, which may differ from each other and even belong to different strains. So the mutation that a researcher discovers does not always mean a great discovery. It could be simply a sequence of another virus.

In this study, we compared the sequence of a presumably mutated influenza virus to several reference viruses and carefully weighed the results: what can actually be considered a mutation in the epitope sequence that determines vaccine failure, and what is statistical or technical error in sequencing?

For the purpose of this study a viral sequence was obtained from a patient who became infected even though he had received the seasonal vaccine.

Materials and Methods

D*ata*

To perform targeted deep sequencing experiments for analyzing the HA genes in a patient's viral sample, an Illumina single-end sequencing run was used. The sequence KF848938.1 (GenBank ID) in fasta format was taken as a reference, SRR1705858, SRR1705859 and SRR1705860 fastq-files from SRA FTP were used as control samples.

Tools

Alignment was performed with BWA-MEM (ver. 0.7.17) on reference KF848938.1, sorted and indexed BAM-file was created by samtools (ver. 1.6, depth limit -d for samtools mpileup was taken as 33000). VarScan (ver. 2.4.3) was used for variant calling, The demonstration of the sequence was performed with IGV. Accurate set of actions and commands can be found in the Supplementary section.

Results

As the result of samtools work we obtained mapped reads for the tested sample (KF848938.1) and three reference samples. Statistical meanings are performed in Table 1.

Table 1. Statistics on reads count

dataset ID	number of reads (abs.)	mapped reads
KF848938.1	361 349	358 032
SRR1705858	256 744	256 500
SRR1705859	233 451	233 251
SRR1705860	250 184	249 888

For reference samples were calculated average and standard deviation of the frequencies. Table 2 gives a look at accurate meanings.

Table 2. Frequency analysis for reference sample

statistics	**SRR1705858**	**SRR1705859**	**SRR1705860**
mean	0,2564912281	0,2369230769	0,2503278689
standard deviation	0,07172594739	0,05237640771	0,07803775183

When we estimated statistical parameters with SNP-variants and frequency that were provided by VarScan, we came to the conclusion that mutations at 72, 117, 774, 999, 1260 locus are unlikely to be responsible for lack of vaccine effect (Table 3). The high frequency values indicate that these mutations were detected in almost all reads of the test sample. On the contrary, intermediate values of the mutation frequency at 307 and 1458 positions indicate that this is a relatively new “acquiring”, which is nevertheless detectable enough to not merge with sequencing errors. (p-value = 0.001)

Table 3. SNP. Mutations in both tested and control samples annotated with red, mutations that more likely append only in test sample annotated with green

location	nucleotide	replacement	frequency	meaning
72	A	G	99.96%	
117	C	T	99.82%	
254	A	G	0.18%	
307	C	T	0.96%	(CCG -> TCG; P -> S)
340	T	C	0.18%	
389	T	C	0.22%	
722	A	G	0.22%	
744	A	G	0.18%	
774	T	C	99.97%	
802	A	G	0.24%	
915	T	C	0.2%	
999	C	T	99.86%	

1043	A	G	0.19%	
1086	A	G	0.21%	
1213	A	G	0.22%	
1260	A	C	99.94%	
1280	T	C	0.18%	
1458	T	C	0.83%	(TAT -> TAC, Y -> Y)
1460	A	G	0.18%	

Mutation at the locus 1458 results in the replacement of the TAT triplet with TAC triplet, and it seems to be synonymic substitution that doesn't lead to amino acid change. Mutation at the locus 307, however, is more interesting. The CCG triplet encoded proline is now replaced by a TCG triplet encoded serine.
According to Muñoz et al. [5], the mutation affected the epitope C of the hemagglutinin protein, which answers the question posed in this study. Visualization with AlphaFold2 was performed after the Discussion section.

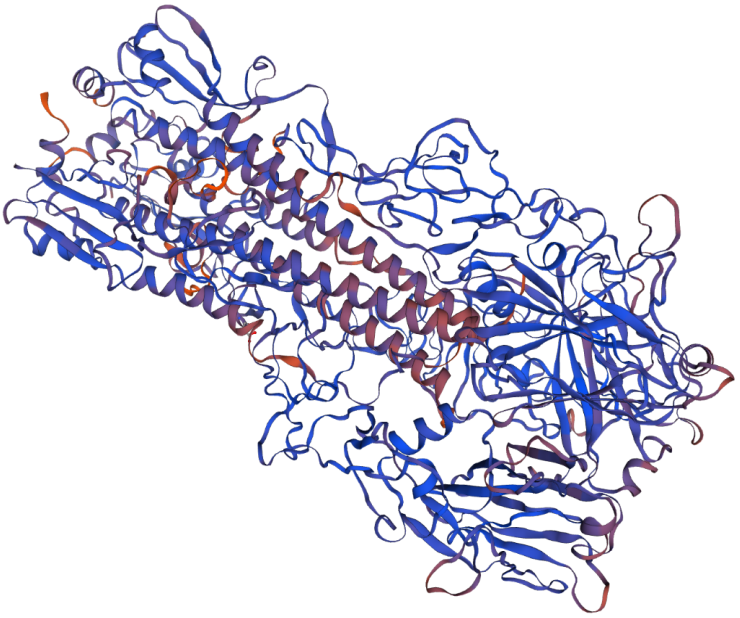
Discussion

A mutation in the epitope C resulted in a change in the protein sequence and complete structure of hemagglutinin. Hemagglutinin is the main component of influenza vaccines; it is the protein that is recognized by immunocompetent cells. The mutated virus is now different from the typical seasonal strain against which the vaccine was created. Therefore even those who have received the vaccine in a given season are at risk of contracting the disease.

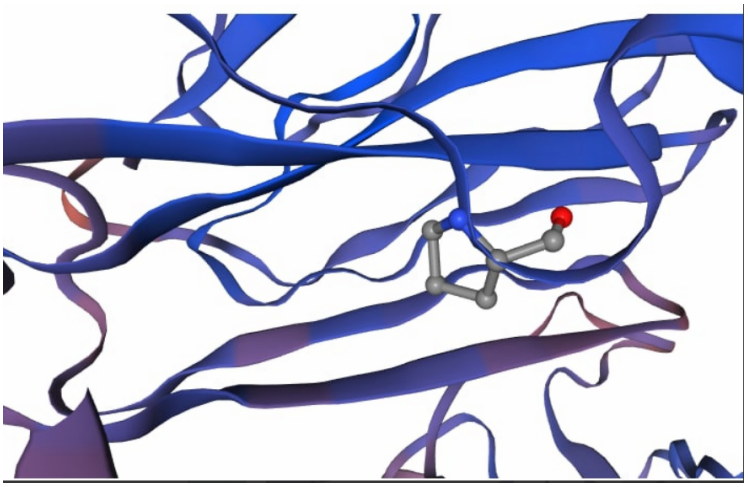
Some changes in HA can render the vaccine completely useless: viruses with new protein sequence will elude immunocompetent cells undetected. To keep up with viruses in this race, we should constantly monitor the nucleotide sequence of the virus for such mutations.

Sequencing errors are a common phenomenon, which is nevertheless inconvenient. in addition to "debugging" to the reference, you can remove the end sections during data analysis (where errors occur most often if sequencing was performed with illumina).

Model based on 4we8.1.C Hemagglutinin:



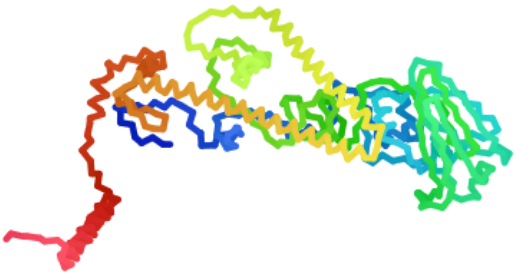
Original proline at the chain:



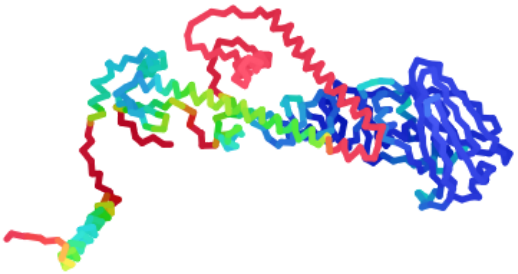
According to AlphaFold2 modeling, Influenza A virus (A/USA/RVD1_H3/2011(H3N2)) segment 4 hemagglutinin (HA) gene, partial cds with the occurred mutation will have the following structure:

Model 1

colored by N→C

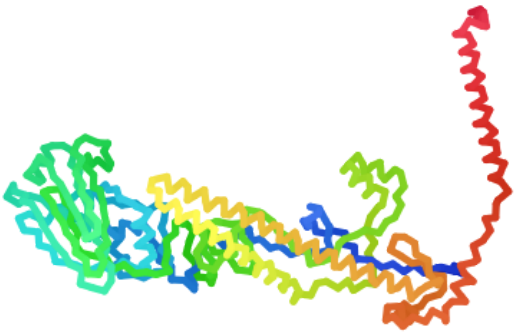


colored by pLDDT

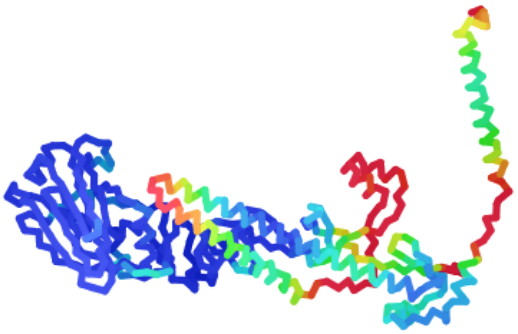


Model 2

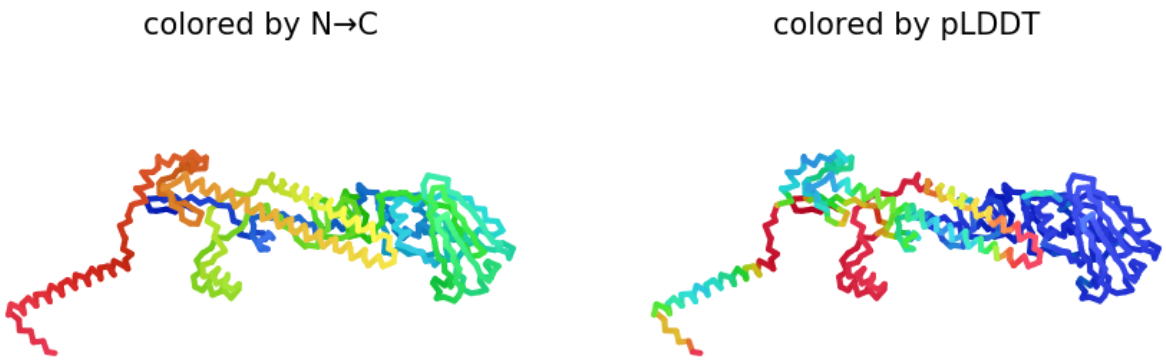
colored by N→C



colored by pLDDT



Model 3



So it won't fold in the same structure.

Literature

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Supplementary

лабжурнал 2