Title: CRISPR Cas9 as a Possible Mechanism to Modify Influenza Viral Mutation

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All images/charts/graphics created by the researcher unless otherwise referenced.
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Introduction

- Influenza virus, also known as Orthomyxoviridae (Velthuis & Fodor, 2016), is a type of single stranded RNA virus.
- Since it has a single stranded RNA genome, the mutation rate is higher than that of a virus with a double stranded DNA genome (Sanjuán & Domingo-Calap, 2016).
- There are three types of Influenza (A, B, & C) that affect humans and 29 subtypes which are classified based on their surface protein, hemagglutinin (HA) or neuraminidase (NA) (Jilani, Jamil, & Siddiqui, 2020).
- According to the Centers for Disease Control and Prevention (2020), within the six months up to April 2020, there were over 39 million cases of the influenza virus in the U.S.A., out of which, over 24 thousand resulted in death.
- The fact that the influenza virus has a high mutation rate makes it more difficult to create an effective vaccine (Shao, Li, Goraya, Wang, &; Chen, (2017).
- CRISPR Cas9, or Clustered Regularly Interspaced Palindromic Repeats is a gene-editing technique, which could potentially be used as a method to modify the Influenza Virus mutations.

Framework

1. Acquire background information on CRISPR Cas9, the Influenza virus and its mechanisms of mutation through articles published on PubMed using the following keywords: CRISPR Cas9, Influenza Virus, Viral Mutations, Viral replication, viral genomes, sequences.
2. Study the subtypes of Influenza A virus and organize them in a table based on the protein that determines the subtype, and the species affected.
3. Calculate the average number of subtypes per species and present in a bar graph.
4. Determine 5 different influenza A subtypes (H7N9, H5N1, H2N2, H1N1, and H3N2) and find the FASTA genetic sequences for their HA and NA segments using NCBI Influenza.
5. Align the 5 HA sequences and the 5 NA sequences using Clustal Omega.
6. Insert the aligned sequences into GeneDoc in order to demonstrate the conservation between the genome of the different subtypes of Influenza A, displaying where the mutations occurred.
7. Analyze the GeneDoc results to determine where CRISPR Cas9 could be applied.

Findings

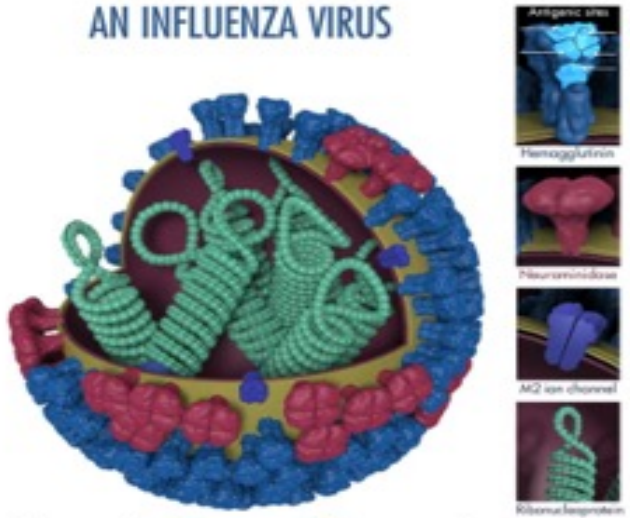

Figure 1. Influenza Virus and surface proteins' structures.
Credit: Center of Disease Control (CDC)
Retrieved from: https://www.cdc.gov/flu/resource-center/freeresources/graphics/images.htm

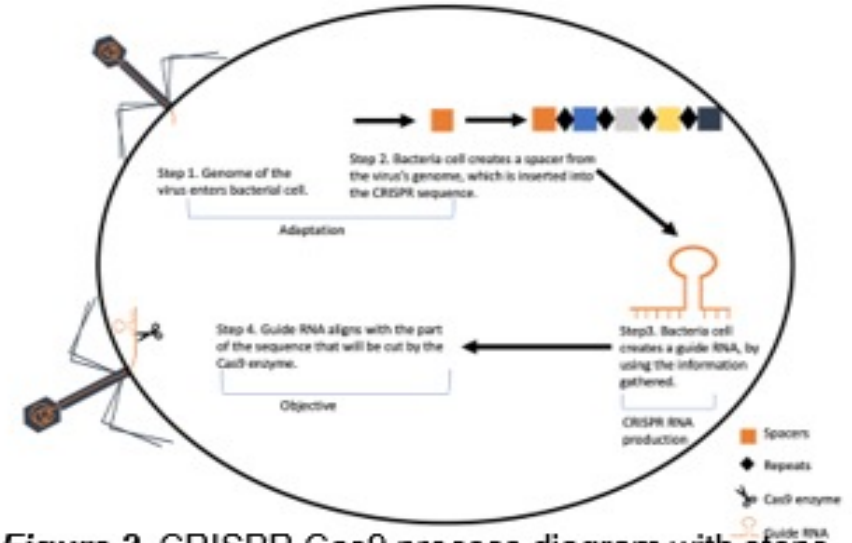

Figure 2. CRISPR Cas9 process diagram with steps.
Credit: Harvard University
Based on: https://sitn.hms.harvard.edu/flash/2014/ crispr-a-game-changing-genetic-engineering-technique/

Class 1	
Type I	**Type III**
Cas5	Cas10
Cas3	
Cas6	

Table 1. Class I CRISPR versions categorized between type I and III.

Class 2			
Type II	**Type IV**	**Type V**	**Type VI**
Cas9	Cas7	Cas12	Cas13
	Cas8	Cpf1	
	Cas6		
	Cas1		
	Cas2		

Table 2. Class II CRISPR versions categorized between type II, IV, V, and VI.

Subtypes of Influenza A by Species Affected			
Hemagglutinin	**Neuraminidase**	**Hemagglutinin**	**Neuraminidase**
HUMANS/ANIMALS		***ANIMALS***	
H1	N1	H4	N3
H2	N2	H8	N4
H3	N6	H11	N5
H5	N7	H12	N10
H6	N8	H13	N11
H7	N9	H14	
H9		H15	
H10		H16	
		H17	
		H18	

Table 3. Influenza A subtypes categorized by surface protein, and species affected.

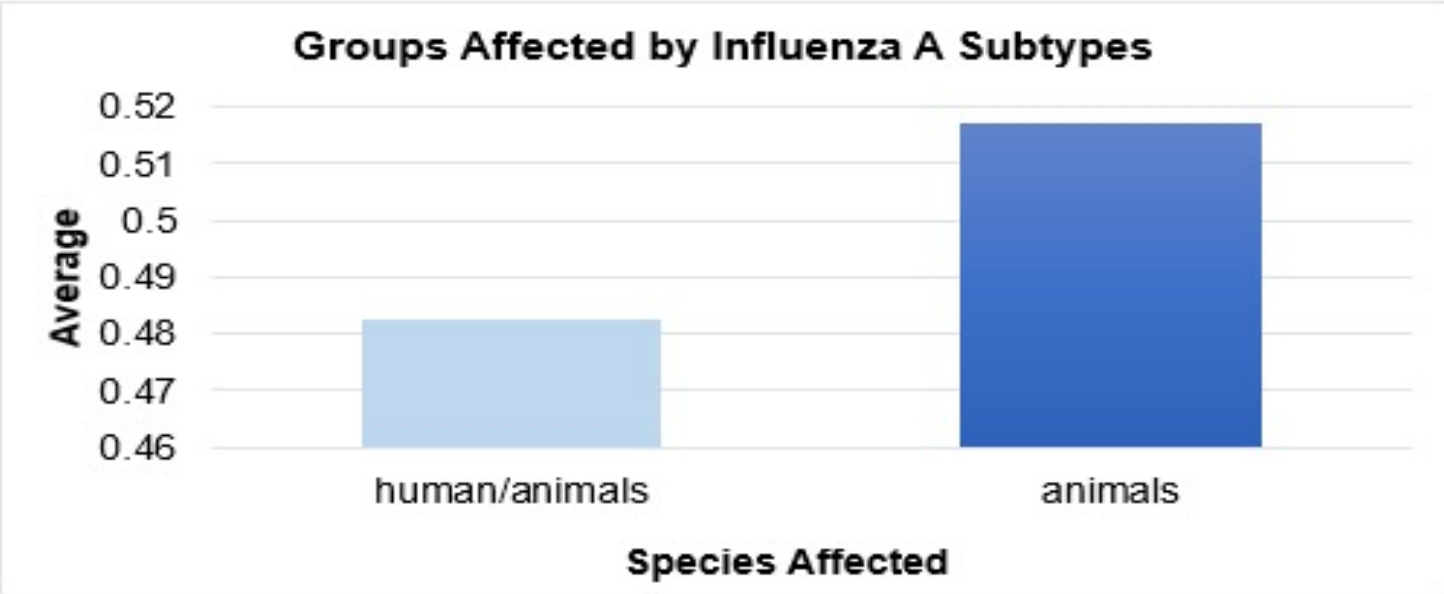

Figure 3. Average of Influenza A subtypes based on groups of species affected.

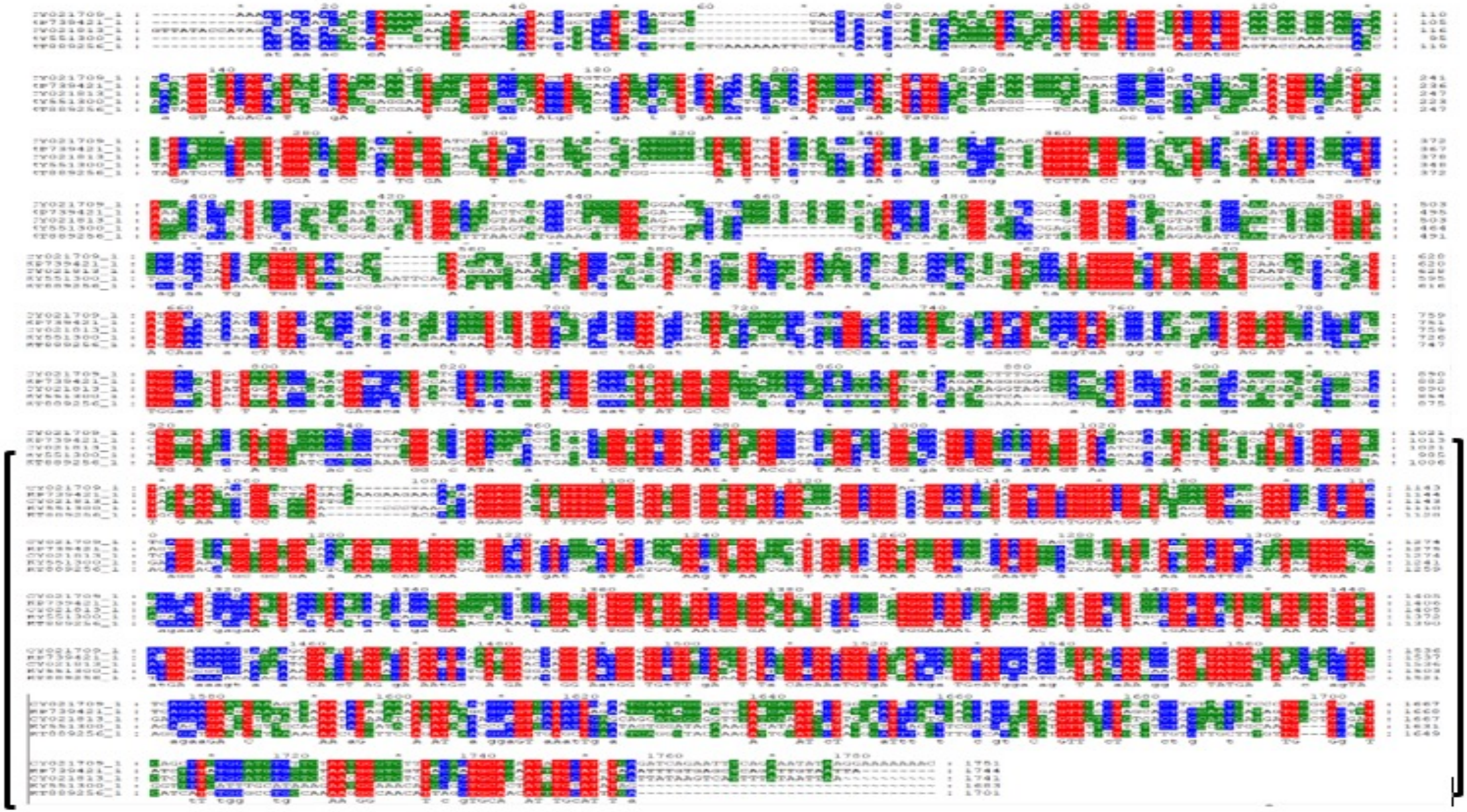

Figure 4. Genetic sequences of HA segment of 5 different Influenza A subtypes. The "blocks" identified with double brackets are those that showed the most conservation from the complete sequence alignment.

Figure 5. Genetic sequences of NA segment of 5 different Influenza A subtypes. The "blocks" identified with double brackets are those that showed the most conservation from the whole sequence alignment.

Discussion

- One of the most recent strains is an influenza A subtype, H1N1, is composed of different segments from four previous strains. Usually, every two or three years a more virulent strain appears. The strains that make up the influenza A H1N1 are: Avian influenza, Human influenza, Eurasian swine virus, North American Swine Virus.
- Within the influenza A type, there are 29 subtypes depending on the presence of HA and NA proteins displayed in Table 3.
- Mutation occurs through the antigenic drift that affects the genes that encode the surface proteins, HA and NA, and the antigenic shift causes new HA or NA proteins to be created. Influenza viruses have 8 gene segments that make up their RNA, encoding for the 12 proteins that make up the virus. RNA polymerase PB2 unit, RNA polymerase PB1 unit, RNA polymerase PA unit, hemagglutinin (HA), nucleoprotein (NP), neuraminidase (NA), matrix (M), and non-structural protein (NS). Antigenic shift and drift affect the hemagglutinin segment and the neuraminidase segment which are seen in Figure 4 and 5.

- The results display the conservation between the genetic sequences of the HA and NA segments of H7N9, H5N1, H2N2, H1N1, and H3N2 subtypes, in order to determine the specific location in the sequence where the mutations occurred.
- As seen in figures 4 and 5, the sequences underwent very noticeable changes, which makes the CRISPR Cas9 application more difficult, since there are many different locations in which mutations occurred.

Conclusions

Based on the data collected, and the identification of the mutations' locations within the virus's genetic material, it is likely that the CRISPR Cas9 mechanism can be applied to the Influenza A virus genome in order to prevent further mutations from occurring. The mechanism would be applied so that it identifies which parts of the genome remained constant, to modify or delete the segments that did not. Ultimately, the prevention of the development of new mutations would limit the number of subtypes that surface throughout the years. Future investigations might include determining if there is another mechanism that can be used to modify a viral mutation, working on using the CRISPR editing method on other genome segments of the virus, that don't undergo antigenic shift and drift, and if CRISPR Cas9 can be used, determine how it can be implemented in the development of a vaccine or treatment for Influenza.

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