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Determination of restriction sites of hemagglutinin, neuraminidase and nucleoprotein encoding genes of h1n1 and h7n9 strains of influenza virus

Abstract

Introduction: Influenza virus (family Orthomyxoviridae) is the causative agent of influenza-an acute, highly contagious disease of the respiratory and gastrointestinal tract. The most important influenza virus virulence factors are surface proteins - hemagglutinin and neuraminidase, as well as nucleoprotein. These substances also have antigenic properties. High variability of the genes which are encoding these proteins leads to the emergence of strains with new antigenic properties every 1-2 years, which allows the virus to overcome strain-specific immunity in the population and to receive epidemic spread. Diagnosis and mapping of the genome of the virus due to its high level of variability requires high-quality and highly sensitive molecular methods, such as the technique of restriction fragment length polymorphism (RFLP). The aim of this work was to determine the restriction sites of hemagglutinin, neuromidinase and nucleoprotein genes and to model the RFLP analysis of Influenza virus using the pathogenic H1N1 and H7N9 strains with the help of bioinformatic methods in silico.

Materials and methods: The restriction fragment length polymorphism assay was performed on the nucleotide sequences of HA, NA, and NP genes encoding hemagglutinin, neuraminidase, and nucleoprotein, H1N1, and H7N9 influenza strains which were taken from the GenBank database of the National Center for Biotechnology (NCBI). The restriction sites were found, their location on the genes was examined, and the nucleotide distances between them were calculated. Restriction enzyme was detected for further performing of RFLP analysis. The RFLP analysis with restrictase to the well-established sites was modeled and the sizes of its products in silico were determined. The studies were performed using VectorNTI-11.

Result: Common and unique RFLP analysis products for HA, NA and NP genes of H1N1 and H7N9 influenza strains have been identified. The distribution of RFLP analysis products by electrophoresis in silico is shown. The possibility of using restriction fragment length polymorphism to identify strains of H1N1 and H7N9 influenza virus was shown.

Keywords: influenza virus, strain H1N1, strain H7N9, hemagglutinin, neuraminidase, nucleoprotein, restriction sites

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Introduction

The influenza virus Influenza virus from the *Orthomyxoviridae* family is one of the most common pathogens for respiratory and gastrointestinal infections. Influenza virus based on antigenic differences between internal nucleoproteins (NP) and surface glycoproteins hemagglutinin (HA), as well as the neuraminidase (NA) enzyme is divided into types A, B, and C.[1-3] The influenza virus has specific features. The high variability of NP, NA and HA leads to the emergence of viruses with new antigenic properties every 1-2 years, which allows it to overcome strain-specific immunity in the population and to receive epidemic spread.[4]

A peculiarity of the genome of the influenza virus is the lack of a mechanism of protection against errors when reading the genetic information that underlies such frequent changes in amino acid sequences of proteins.[5,6] Mutations that occur in the course of antigenic drift and shift (nucleotide substitutions, deletions, insertions) go unnoticed. Viruses with such changes in NP, NA, and HA proteins are fixed in the circulation, which allow it to overcome the strain-specific immunity available in the majority of the population.[7,8]

In this regard, almost every year, the replacement of strains of viruses that are part of the flu vaccine is required. All this makes relevant the development of high-quality mapping techniques and diagnostics of new and long isolated strains of Influenza virus.[9,10] One of the most sensitive methods of analysis of the genome of Influenza virus is the restriction fragment length polymorphism (RFLP) method, which reveals the presence or absence of mutations in Influenza virus strains and requires a minimal amount of viral material.[11]

The purpose of this work was to determine the restriction and simulation sites for RFLR analysis of Influenza virus using the H1N1 and H7N9 strains.

Materials and methods

For analysis of restriction fragments of HA, NA, and NP genes encoding NA, NA, and NP proteins, H1N1 and H7N9 strains of influenza virus, all nucleotide sequence data available in the GenBank database of the National Center for Biotechnology Information (NCBI) were used. genes of the studied strains. Search of restriction sites on the obtained nucleotide sequences of genes HA, NA and NP,

modeling RFLP analysis, determining their size and electrophoresis of RFLF products in silico were performed using VectorNTI-11.

Results and discussion

The VectorNTI-11 program identified the restriction sites of the HA, NA, and NP genes of H1N1 and H7N9 strains of influenza A virus and obtained the corresponding restriction enzyme (Table 1).

Figures 1-6 show schematics of the studied genes for H1N1 and H7N9 influenza strains with restriction sites.

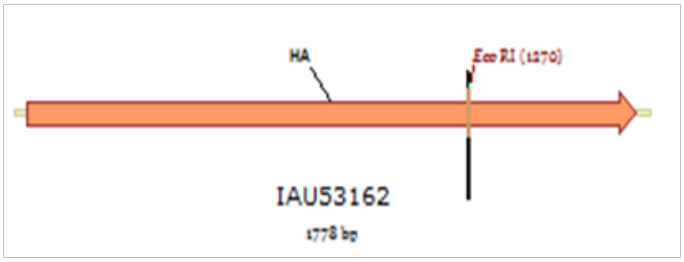

Figure 1 Scheme of the HA gene of strain H1N1 (5 '- 3'). The restriction name is indicated by the restriction site.

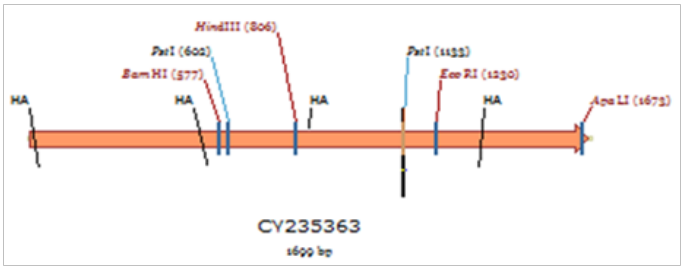

Figure 2 Scheme of the HA gene of strain H7N9 (5 '- 3'). The restriction name is indicated by the restriction site.

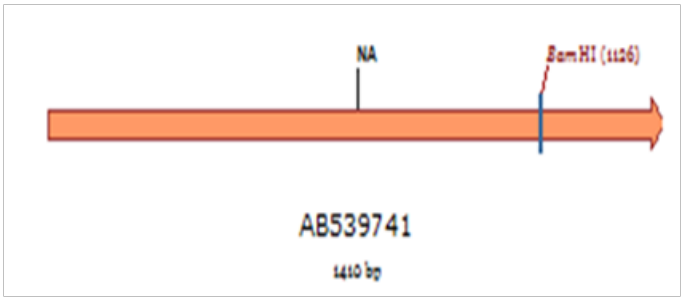

Figure 3 Scheme of the NA gene of strain H1N1 (5 '- 3'). The restriction name is indicated by the restriction site.

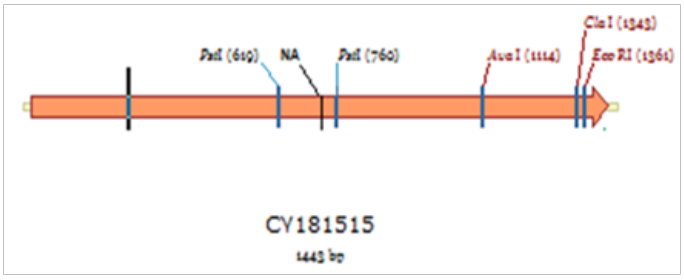

Figure 4 Scheme of the NA gene of strain H7N9 (5 '- 3'). The restriction name is indicated by the restriction site.

Thus, the study revealed the restriction sites of genes HA, NA and NP strains of H1N1 and H7N9 influenza virus. The HA and NA genes of strain H1N1 have only one restriction site, which makes it impossible to further use RFLP analysis to diagnose these strains for these genes. Products of RFLP analysis of HA, NA, and NP genes of H1N1 and H7N9 strains of influenza A virus. VectorNTI-11 program performed RFLP analysis of influenza virus HA, NA, and NP strains of strains at selected restriction sites (Table 2).

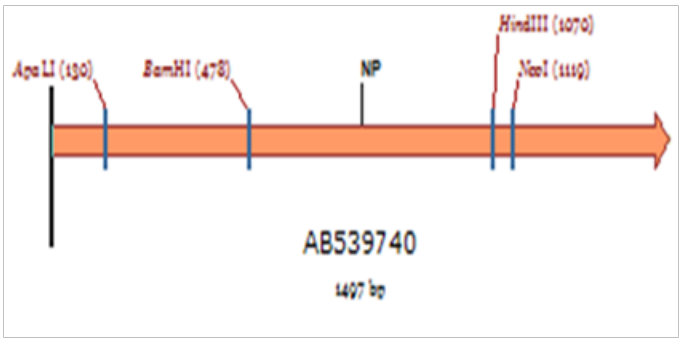

Figure 5 Scheme of the NP gene of strain H1N1 (5 '- 3'). The restriction name is indicated by the restriction site.

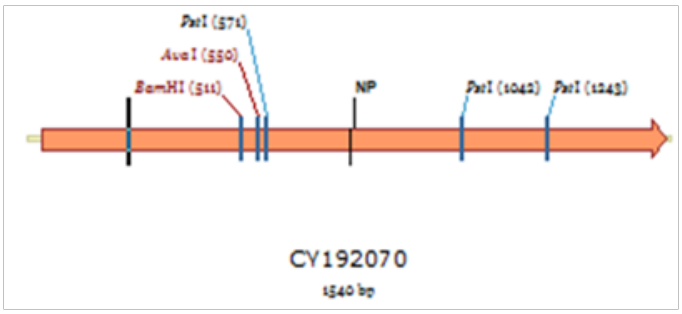

Figure 6 Scheme of the NP gene of strain H7N9 (5 '- 3'). The restriction name is indicated by the restriction site.

Thus, the size of the products of the RFLP analysis of the HA, NA and NP genes of the H1N1 and H7N9 strains of influenza virus was determined. Each of the detected RFLR fragments is unique in length and can be used to identify the HA, NA, and NP genes of strain H7N9 and the NP gene of strain H7N9.

As a result of the selection of restriction sites using VectorNTI-11, RFLP analysis was performed in silico and the possibility of identification of strain H7N9 by HA, NA and NP genes and strain H1N1 by NP gene was demonstrated.

Scheme of identification of RFLP products by good restriction sites of influenza virus strains H1N1 and H7N9 by electrophoresis in silico is shown in Figure 7.

1 - quality control of the reaction (RFLR-mixture without viral RNA); 2 - product RFLP gene analysis for strain H1N1; 3 - product RFLP gene analysis for strain H7N9; 4 is a product of the RFLP analysis of the NA gene of strain H1N1; 5 is a product of the RFLP analysis of the NA gene of strain H7N9; 6 is a product of the RFLP analysis of the HP gene of strain H1N1; 7 is a product of the RFLP analysis of the HP gene of strain H7N9; 8 - DNA marker molecular weight of 100 n.p. Ladder.

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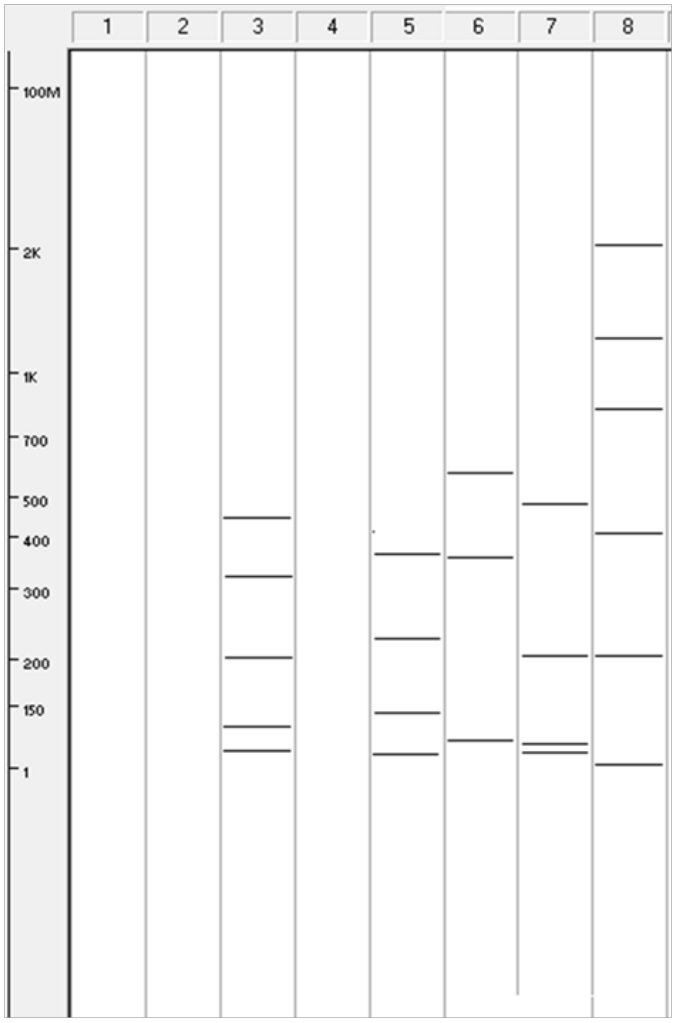

Figure 7 Scheme of identification of products RFLP analysis of strains H1N1 and H7N9 of influenza virus by electrophoresis *in silico*.

Table 1 Sites of restriction of HA, NA and NP genes of H1N1 and H7N9 strains of influenza virus

Gene	Strain	Restriction site	Number of restriction sites of this type
HA	H1N1	EcoR1	1
		PstI	2
	H7N9	BamHI	1
		HindIII	1
		EcoR1	1
		ApaLI	1
NA	H1N1	BamHI	1
		PstI	2
	H7N9	AvaI	1
		ClaI	1
		EcoR1	1
NP	H1N1	ApaLI	1
		BamHI	1
		HindIII	1
	H7N9	NcoI	1
		BamHI	1
		AvaI	1
		PstI	3

Table Continued

Table 2 Results of RFLP analysis of HA, NA and NP genes of H1N1 and H7N9 strains of influenza virus

Gene	Strain	The size of the RFLP analysis products (n.p.)
HA	H1N1	-
	H7N9	25 – 27, 97 – 101, 204 – 210, 327 – 330, 443 – 449
NA	H1N1	-
	H7N9	18, 141 – 143, 229 – 232, 354 – 359
NP	H1N1	49 – 50, 348 – 350, 592 – 599
	H7N9	21, 39, 201 – 203, 471 – 480

Conclusion

According to the results of the study, restriction sites and their location on the genes HA, NA and NP of strains H1N1 and H7N9 of influenza virus were determined. RFLP-analysis with restrictase to good restriction sites and electrophoresis of its products in silico was performed. The unique lengths for each of the studied strains of RFLP fragments are determined, which can be further used to identify the HA, NA, and NP genes of strain H7N9 and the NP gene of strain H7N9, as well as for mapping of all tested genes of strains H1N1 and H7N9.

Acknowledgments

None.

Conflicts of interest

Authors declare that there is no conflict of interest.

References

1. Allison AB, Ballard JR, Tesh RB, et al. Cyclic Avian Mass Mortality in the Northeastern United States Is Associated with a Novel Orthomyxovirus. *J Virol*. 2015;89(2):1389–1403.
2. Dukhovlinov I, Al-Shekhadat R, Fedorova E, et al. Study of immunogenicity of recombinant proteins based on hemagglutinin and neuraminidase conservative epitopes of Influenza A virus. *Med Sci Monit Basic Res*. 2013;19:221–227.
3. Wu C, Lin C, Tsai T, et al. Influenza A surface glycosylation and vaccine design. *Proc Natl Acad Sci U S A*. 2017;114(2):280–285.
4. Webster RG, Bean WJ, Gorman OT, et al. Evolution and ecology of influenza A viruses. *Microbiol Rev*. 1992;56 (1):152–179.
5. Tada T, Suzuki K, Sakurai Y, et al. Emergence of Avian Influenza Viruses with Enhanced Transcription Activity by a Single Amino Acid Substitution in the Nucleoprotein during Replication in Chicken Brains. *J Virol*. 2011;85(19):10354–10363.

6. Taubenberger JK, Kash JC. Influenza Virus Evolution, Host Adaptation and Pandemic Formation. *Cell Host Microbe*. 2010;7(6):440–451.

7. Ozawa M, Kawaoka Y. Crosstalk between animal and human influenza viruses. *Annu Rev Anim Biosci*. 2013;1:21–42.

8. Staats CB, Webster RG, Webby RJ. Diversity of influenza viruses in swine and the emergence of a novel human pandemic influenza A (H1N1). *Influenza Other Respir Viruses*. 2009;3(5):207–213.

9. Cooper LA, Subbarao K. A Simple Restriction Fragment Length Polymorphism-Based Strategy That Can Distinguish the Internal Genes of Human H1N1, H3N2, and H5N1 Influenza A Viruses. *J Clin Microbiol*. 2000;38(7):2579–2583.

10. Zhang X, Kong W, Ashraf S, et al. A one-plasmid system to generate influenza virus in cultured chicken cells for potential use in influenza vaccine. J Virol. 2009;83(18):9296–9303.

11. Klimov AJ, Cox NJ. PCR restriction analysis of genome composition and stability of cold-adapted reassortant live influenza vaccines. *J Virol Methods*. 1995;52(1-2):41–49.

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