Whole genomic analysis of two influenza H3N2 virus strains isolated from Qinghai, China

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Abstract

Background Influenza H3N2 virus has a faster evolution rate than other types of influenza viruses. This study was performed to better understand the molecular evolution of influenza H3N2 in Qinghai Province, China in 2017.

Methods Complete sequences of eight gene segments of two influenza H3N2 isolates in 2017 in Qinghai Province were sequenced and analyzed by MEGA 6.06 software.

Results Phylogenetic analysis showed that two Qinghai H3N2 isolates were relatively close to the 2016–2017 vaccine strain, 3C.2a-A/Hong Kong/4801/2014. In HA protein, compared with the 2015-2016 WHO recommended vaccine strain A/Switzerland/971 5293/2013, six amino acid substitutions were observed in epitopes A and B in Qinghai isolates in 2017, however, only two amino acid substitutions were observed in epitopes A and B in Qinghai isolates compared with the A/Hong Kong/4801/2014, which indicated 2016-2017 vaccine strain might have a better protection against the strains circulating in Qinghai Province in 2017. Besides, amino acid substitution of K160T at the glycosylation site of HA and H75P in PB1-F2 in the two Qinghai isolates might affect the antibodies binding ability and the virulence of influenza virus. And there was no key amino acid substitution in the key sites of segment NA, M, NP, NS, PA and PB2.

Conclusions The presence of several antigenic site mutations in Qinghai H3N2 isolates confirms the evolution of circulating H3N2 strains. Enhancing the surveillance of influenza epidemic by whole genome sequencing is important to monitor whether the selected vaccine strains are protective against the circulating strains in Qinghai Province.

Introduction

Influenza virus is an enveloped, single-stranded RNA virus with negative strand, containing eight fragments, haemagglutinin (HA), neuraminidase (NA), nucleoprotein (NP), non-structural (NS), matrix (M), polymerase acidic (PA), polymerase basic 1 (PB1) and polymerase basic 2 (PB2). By constantly changing the antigenicity, influenza virus can evade the identification and elimination of host specific immunity, and thus continuously causes epidemics which is one of the important public health issues worldwide[1]. H3N2 subtype of influenza virus was first identified in 1968 and caused the third flu pandemics of 20th century [2]. Owing to the rapid evolution, influenza H3N2 virus have always been active in the crowds. Therefore, it is important to monitor and explore the variation rules of influenza virus for the prediction of influenza outbreak and the screening of influenza vaccine.

Influenza vaccines have been developed to protect the general population from developing severe disease[3]. Considering the rapid viral antigenic evolution of influenza virus, vaccines need to be reformulated periodically [4]. Twice a year, the World Health Organization (WHO) provides recommendations on influenza vaccines strains for the upcoming influenza season, for southern and northern hemispheres respectively. The application of complete influenza virus genomes is deepening our understanding of influenza evolutionary characteristics and promoting the selection of vaccine strains.

In this study, we firstly performed the whole genome sequencing of influenza H3N2 virus in Qinghai Province, located in the northwest of China. We analyzed eight gene segments of two influenza A/H3N2 virus strains circulating in 2017 and compared them with those of the WHO recommended vaccine strains and with some other H3N2 strains circulating in other areas at the same time from Gen Bank and GISAID, which benefit us for understanding the molecular epidemic characteristics of influenza H3N2 virus in Qinghai Province in 2017 and assessing the protective effect of WHO recommended vaccine strains in this area.

1. Material And Methods

1.1 Virus

Two strains of influenza H3N2 virus involved in this study were selected randomly, which were isolated by Madin-Darby canine kidney cells (MDCK), and identified by hemagglutination (HA) test and hemagglutination inhibition (HI) test [5, 6].

1.2 Whole genome sequencing

Viral RNA was extracted by using RNA/DNA extraction kit (ZTLJB-Y64) on nucleic acid extraction system NP968 according to manufacturer's instructions (Tianlong Bio-technology Co., Ltd., China). PCR was performed by using QIAGEN One-step RT-PCR kit (QIAGEN, Germany) according to the manufacturer's instructions, the primers of HA, NA, NP, NS, M, PA, PB1 and PB2 gene were designed by Yi-Mo Deng et al [7] and synthesized in Sangon Biotech (Shanghai, China). Then the mixture was run using the following program: 60°C1min, 42°C10min, 50°C30min, 95°C15min, (94°C30s, 50°C30s, 72°C1min)×35 cycles, 72°C10min. The PCR products were identified using 1.5% agarose gel electrophoresis and then sent to Sangon Biotech (Shanghai, China) for Sanger sequencing. Genbank accession numbers range from MN533932 to MN533947 for virus isolate sequences.

1.3 Sequence analysis

The nucleotide sequences were spliced by using SeqMan, analyzed by using BioEdit version 7.0.4.1 and the NCBI BLAST software (<http://blast.ncbi.nlm.nih.gov/>). MEGA 6.06 software was used for phylogenetic analysis of the aligned sequences. The phylogenetic tree was generated using the neighbor-joining algorithm. The credibility of the phylogenetic trees was tested by applying a bootstrap test with 1000 replications. WHO recommended influenza vaccine strains from 2010 to 2017 and some influenza isolates from other areas during 2016 to 2017 were obtained from Genbank and GISAID.

2. Results

2.1 Phylogenetic analysis

The phylogenetic tree of nucleotide sequences of eight gene fragments of influenza H3N2 virus was constructed by NJ method, the WHO recommended vaccine strain for northern hemispheres during 2010–2017: 1-A/Perth/16/2009 (2010–2012), 3C.1-A/Victoria/361/2011 (2012–2014), 3C.1-A/Texas/50/2012 (2014–2015), 3C.3a-A/Switzerland/9715293/2013 (2015–2016), and 3C.2a-A/Hong Kong/4801/2014 (2016–2017) were chose as the reference strains. Phylogenetic analysis of HA, NA, M and PA gene showed that two Qinghai isolates in 2017 clustered with the 2016–2017 vaccine strain 3C.2a-A/Hong Kong/4801/2014, phylogenetic analysis of NS, PB1 and PB2 gene showed that two Qinghai isolates clustered with the 2016–2017 vaccine strain 3C.2a-A/Hong Kong/4801/2014 and 2015–2016 vaccine strain 3C.3a-A/Switzerland/9715 293/2013. In the phylogenetic tree of NP gene, the Qinghai isolates clustered with the isolates from other areas at the same period, but did not cluster with any vaccine strains (Figure 1).

2.2 Amino acid sites analysis

2.2.1 HA protein

HA protein plays a key role in determining the virulence of influenza viruses, which is closely related to the prevalence of influenza, and is the molecular basis of antigenic variation of influenza viruses [8]. Compared with the HA gene of 2015–2016 vaccine strain A/Switzerland/9715293/2013, a total of 16 amino acid substitutions were observed in

Qinghai isolates in 2017. Compared with the HA gene of 2016–2017 vaccine strain A/Hong Kong/4801/2014, a total of 7 amino acid substitutions were observed in Qinghai isolates in 2017 (Table 1).

HA protein was spliced into two subunits HA1 and HA2 at site 329, showing characteristic differences in splicing sites between highly pathogenic influenza viruses and low-pathogenic influenza viruses. In our study, the splicing site of the two Qinghai isolates was PEKQTR↓G, which were typical low-pathogenic influenza viruses. HA protein have 5 epitopes, all of which are located in the HA1 subunit, including 129 sites of A, B, C, D and E. Among them, epitopes A and B are the most important, which can induce the protective antibodies[9]. Compared with the 2015–2016 vaccine strain A/Switzerland/9715293/2013, the amino acid substitution of R140I, G142K, N144S, S159Y, K160T and P194L were observed in epitopes A and B in Qinghai isolates. Compared with the 2016–2017 vaccine strain A/Hong Kong /4801 /2014, only the amino acid substitution of R142K and K160T were observed in epitopes A and B in Qinghai isolates (Table 1).

Receptor binding sites of influenza H3N2 virus contain 134–138 residues, 224–228 residues and 6 key sites of 98, 153, 190, 194, 183 and 155[10]. Compared with the 2015–2016 vaccine strain A/Switzerland/9715293/2013, amino acid substitution of S138A was observed in the Qinghai isolate A/QH/219/2017, and P194L was observed in the two Qinghai isolates. Compared with the 2016–2017 vaccine strain A/Hong Kong/4801/2014, amino acid substitution of S138A was observed in the Qinghai isolate A/QH/245/2017.

The 2015–2016 vaccine strain A/Switzerland/9715293/2013 has 11 glycosylation sites, located at sites 8, 22, 38, 45, 63, 122, 133, 165, 246, 285 and 483, amino acid substitution of A128T and K160T were observed in Qinghai isolates in 2017, and two glycosylation sites at sites 126 and 158 were added. Compared with the 2015–2016 vaccine strain A/Switzerland/9715293/2013, an additional glycosylation site at site 126 appeared in 2016–2017 vaccine strain A/Hong Kong/4801/2014. Compared with these sites, Qinghai isolates added a new glycosylation site at site 158. In addition, analysis of disulfide bonds at amino acid sites 14, 52, 64, 76, 97, 137, 139, 277, 281 and 305 showed no amino acid substitution in Qinghai isolates.

Table 1 Amino acid substitution of HA gene of influenza H3N2 viruses in Qinghai Province in 2017

Strain	Amino acid substitution																
	3	96	128	131	138	140	142	144	159	160	186	194	239	261	311	326	489
A/Switzerland/9715293/2013	L	N	A	T	S	R	G	N	S	K	V	P	P	R	Q	R	D
A/Hong Kong/4801/2014	I	S	T	T	A	I	R	S	Y	K	G	L	P	R	H	K	N
A/QH/219/2017	I	N	T	K	A	I	K	S	Y	T	G	L	S	Q	H	K	N
A/QH/245/2017	I	N	T	K	S	I	K	S	Y	T	G	L	P	Q	H	K	N

2.2.2 NA protein

NA protein is one of the surface glycoproteins of influenza viruses. It can cleave sialic acid at the end of the glycoside chain of HA protein on the surface of host cells and newly synthesized virion, which could assist the mature influenza viruses to escape from host cells and infect new cells [11]. Compared with the NA gene of 2015–2016 vaccine strain A/Switzerland/9715293/2013, a total of 7 amino acid substitutions occurred in Qinghai isolates in 2017. Compared with the NA gene of 2016–2017 vaccine strain A/Hong Kong/4801/2014, a total of 6 amino acid substitutions occurred in Qinghai isolates in 2017 (Table 2).

NA protein contains 7 epitopes, all of which are located in the head region, including amino acids sites of 153, 197–199, 328–336, 339–347, 367–370, 400–403 and 431–434, respectively [12], and there were no amino acid substitution in Qinghai isolates. And there were 8 potential glycosylation sites in NA, which were located at sites 61, 70, 86, 146, 200, 234, 329 and 367, respectively, and amino acid substitution of S247T were observed in Qinghai isolates, which added a new glycosylation site at site 245. NA enzyme activity sites contain catalytic sites R118, D151, R152, R224, E276, R292,

R371 and Y406, as well as frame sites E119, R156, W178, S179, D/N198, I222, E227, H274, E277, N294 and E425[13]. Sequence alignment results showed that the NA enzyme activity site of influenza H3N2 virus in this study was conserved and no amino acid substitution was observed.

Table 2 Amino acid substitution of NA gene of influenza H3N2 viruses in Qinghai Province in 2017

Strain	Amino acid substitution						
	231	245	247	267	380	392	468
A/Switzerland/9715293/2013	I	S	S	T	I	T	P
A/Hong Kong/4801/2014	V	S	S	T	I	T	P
A/QH/219/2017	V	N	T	K	V	T	H
A/QH/245/2017	V	N	T	K	V	I	H

2.2.3 M protein

Key amino acid sites of M1 protein contain RNA binding regions and localization signal regions at sites 101–105, and potential zinc finger structure motifs at sites 148–162 [14], and no amino acid substitution was observed in Qinghai isolates.

The M2 protein is the target protein of the anti-influenza drug amantadine, in which the core sites of 37H and 41W, and sites 26, 27, 30, 31 and 34 are closely related to the therapeutic effect of antiviral drugs [15], and no key amino acid substitution was observed in Qinghai isolates.

2.2.4 NP and NS protein

NP protein is the main structural protein of influenza virus, which amino acids sites 408–419 are the key regions, and 6 amino acid sites, including 16, 33, 100, 136, 283 and 313, are correlated with host specificity[16]. There were no key amino acid substitution was observed in Qinghai isolates.

The core amino acids sites of the N-terminal RNA binding domain of NS1 protein are sites 19–38, and the core amino acids sites of the C-terminal effector domain are sites 134–161. And if the terminal sequence of NS1 protein has ESEV or EPEV motif of binding to the PDZ domain, which would be a newly discovered virulence factor of influenza virus [17]. In this study, there were no key amino acid substitution was observed in Qinghai isolates. and the amino acid terminals sequence were RSEV, which indicated that they had no ability to bind PDZ domain.

2.2.5 PA, PB1 and PB2 protein

Influenza virus polymerase is a heterotrimer consisting of three proteins (PA, PB1, and PB2). Among them, sites 55, 100, 382 and 552 are host-specific sites of PA protein, and sites 199, 475, 567, 627 and 702 are host-specific related sites of PB2 protein, and no key amino acid substitution was observed in Qinghai isolates [18]. PB1-F2 protein is an important virulence factor of influenza virus, and its mitochondrial target sequence is located at sites 69–82, while sites 63–75 is related to mitochondrial localization [19]. In this study, amino acid substitution of H75P were observed in Qinghai isolates, which might have some influence on the virulence of influenza virus.

3. Discussion

Influenza H3N2 virus has a faster evolution rate than other types of influenza viruses, and a large number of new mutant strains have been developed since 1968. In this study, two influenza H3N2 virus strains circulating in Qinghai Province in 2017 are relatively close to 2016–2017 vaccine strain 3C.2a-A/Hong Kong/4801/2014. Previous study showed that a typical influenza A virus variant should have more than 4 amino acid mutations in the HA1 epitope and involve 2–3 epitopes [20, 21]. In this study, compared with the 2015–2016 vaccine strain

A/Switzerland/9715293/2013, the amino acid substitution of R140I, G142K, N144S, S159Y, K160T and P194L were observed in epitopes A and B in Qinghai isolates in 2017, which indicated the significant antigenicity variation. However, compared with the 2016–2017 vaccine strain A/Hong Kong/4801/2014, only amino acid substitution of R142K and K160T were observed in epitope A and B in Qinghai isolates in 2017, which suggest no significant antigenicity variation. The change of glycosylation sites has some influence on the antigenicity and biological characteristics of the virus [22]. In this study, amino acid substitution of A128T and K160T added two glycosylation sites in HA in the Qinghai isolates compared with the 2015–2016 vaccine strain A/Switzerland/9715293/2013, and amino acid substitution of K160T added one glycosylation site in HA in the Qinghai isolates compared with the 2016–2017 vaccine strain A/Hong Kong/4801/2014. Amino acid substitution of K160T was located in epitopes B, which might affect the binding ability of antibodies.

NA protein is a kind of glycoprotein, as one of the flu drug targets, distributed on the influenza virus envelope. Amino acid substitution in the epitope may change virus antigenic, amino acid substitution at enzyme activity center may affect the combination between NA enzyme inhibitor and the influenza virus, and amino acid substitution at glycosylation and disulfide sites may affect the NA protein structure [23, 24]. In this study, no amino acid substitution were observed in the two Qinghai isolates at the key sites of epitopes, enzyme activity center and disulfide bond in NA protein, the amino acid substitution of S247T resulted in an additional glycosylation site at site 245, which effect needs further study. In addition, amino acid substitution of H75P in PB1-F2 protein may have certain influence on the virulence of influenza virus. And, the fragments of NP, NS, M, PA and PB2 were relatively conserved, and no amino acid mutation observed at the key sites.

Combined with the results of phylogenetic analysis and amino acid sequence analysis, it was suggested that the 2016–2017 vaccine strain had better protective effect against influenza H3N2 virus circulating in Qinghai province in 2017 compared with that of 2015–2016 vaccine strain A/Switzerland/9715293/2013, which may be the reason for the reduced epidemic intensity of influenza H3N2 virus in Qinghai Province in 2017 compared with that in 2016. In the future work, we should strengthen the pathogenic surveillance of influenza virus by whole genomic sequencing, which benefit for finding new strains as early as possible, and dealing with influenza pandemic.

4. Conclusions

Influenza H3N2 virus has evolved rapidly, which epidemic is a long-term threat to human health, and vaccination is the most effective way to prevent the infection. Exploring the characterization of genetic and antigenic evolution of influenza H3N2 virus is essential to formulating effective vaccine strategies. Periodic assessment and replacement of vaccine strains are important for influenza pandemic prevention and control.

Abbreviations

HA, haemagglutinin; NA, neuraminidase; NP, nucleoprotein; NS, non-structural; M, matrix; PA, polymerase acidic; PB1, polymerase basic 1; PB2, polymerase basic 2; MDCK, Madin-Darby canine kidney cells

Declarations

Ethical approval and consent to participate

This study was approved by the Ethics Committee of Qinghai Center for Disease Control and Prevention, the related procedures were performed according to the guideline of National Influenza Surveillance Program (Edition 2017). The specimen was collected from nostril, posterior pharyngeal wall and bilateral tonsils by using sterile swabs gently, which

was safety and non-invasive. Before the sample collection, all the subjects or their guardian were informed the purpose of this study, and signed the informed consent.

Consent for publication

All authors of this manuscript declare that we have approved the submission and publication of this manuscript.

Availability of supporting data

Not applicable.

Competing interests

The authors declare no competing interests.

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Authors’ contributions

HXR designed the study, performed sequence alignments and drafted the manuscript. Viral nucleic acids were detected by HL and>NNL, and viral isolation was performed by HXR, YJL and SCZ. JY designed the study and revised the manuscript.

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Figures

Figure 1

Phylogenetic tree of eight segments of influenza virus H3N2. Black dot represent Qinghai isolates, black circle represent strains from other areas of the same period, black triangle represent the northern hemisphere vaccine strains recommended by WHO.