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Diversity of the RNA Polymerase in the H7N9 Influenza A Virus

Jing Li, Wenjun Liu*

CAS Key Laboratory of Pathogenic Microbiology and Immunology, Institute of Microbiology, Chinese Academy of Sciences, Beijing, China
Email: lj418@163.com, *liuwj@im.ac.cn

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Abstract

A novel influenza virus of the H7N9 subtype has infected more than 350 people in China since 19 February 2013. Evolutionary analysis indicates that the virus is a reassortant originated from H7, N9 and H9N2 avian influenza viruses, and bears some amino acids associated with mammalian receptor binding, raising concern over the possibility of a new influenza pandemic. Besides HA and NA, the mutation of the polymerase is known to have an important role in virulence, host adaptation and transmissibility in mammalians. In this article, the annotation of the polymerase protein domain associated with molecular function has been highlighted, suggesting the combination of RNA polymerase of H7N9 viruses is still not stable for host adaptation. In addition, the mutation hallmarks in polymerase gene of H7N9 are compared, providing the potential determinants of the evolution in the H7N9 influenza A virus.

Keywords

Diversity, RNA Polymerase, H7N9 Influenza A Virus

Since Feb 19, 2013, when the initial patient infected with the novel influenza A H7N9 virus from an avian source showed symptoms, 350 laboratory-confirmed cases including more than 50 deaths had been reported in mainland China as of June 8, 2015 (http://www.who.int/csr/don/2013_08_11/en/index.html). After nearly 70 days' quiescence, the cases of human infection with H7N9 virus re-emerged in Zhejiang and Guangdong Province [1]. This virus seems to exhibit low pathogenicity in birds, by contrast with the severe disease that occurs in human beings [2]. Another notable feature of H7N9 is the rapid accumulation of laboratory-confirmed cases of infection in human being, even though phylogenetic and epidemiological evidence suggests that transmission is mainly zoonotic [3]-[7]. This has raised global concerns regarding this novel avian-origin AIV.

Based on phylogenetic analysis of isolates from human and domestic birds, it has been proposed that the

*Corresponding author.

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H7N9 viruses responsible for this outbreak are novel reassortants. Their haemagglutinin (HA) and neuraminidase (NA) genes probably originated from Eurasian avian influenza viruses; the internal genes are derived from avian H9N2 viruses that circulated recently in China [8] [9]. The influenza virus polymerase complex, which is a heterotrimer formed by the PB1, PB2, and PA subunits, is a major determinant of species specificity, transmission, and pathogenesis [10] [11]. In particular, three genetic substitutions K627E, N701D, and R591Q in the PB2 segment of influenza virus have been reported to affect host cell tropism [12]-[14]. A single substitution of glutamate to lysine at the residue 627 (E627K) facilitates the adaption of H5N1 and other avian influenza viruses to mammals and increases transmission and pathogenesis in humans, mice, ferrets, and guinea pigs [14] [15].

To address the possibility that the novel H7N9 virus can better adapt to humans, we analyzed and compared the genomes of H7N9 viruses isolated form fatal cases with those of relevant H7N9 viruses. We particularly focus on the polymerase genes which are more determinant to over the host adaptation and the species barriers.

The genome gene sequence of A/Shanghai/1/2013 was used as a reference sequence to compute the amino acid identities for other polymerase gene sequences. It is observed that other 20 strains all display the highest similarity value. The median identity is range from 98.3% to 98.6% (referred as PB1, PB2 and PA).

Of the three P proteins, PB1 is the best characterized functionally. Biochemical and structural analyses recognize PB1 as responsible for RNA chain elongation [16]. PB1 contains amino acid motifs common to all RNA-dependent RNA polymerases and RNA-dependent DNA polymerases [17]. Mutations within these motifs render the complex inactive for transcription and replication in tissue culture cells [18] [19]. The previous study has showed that PB1 I368V is correlated with H5N1 virus airborne transmission among ferrets [20] [21]. In accordance with this deduction, except Shanghai/1 and Environment/Wuxi/1, all 19 strains H7N9 viruses analyzed here encode PB1-368V. Aside from this position, the residue I to V mutation also found in 164, 528, 637 and 728 positions. PB1-F2, which is encoded on a + 1 reading frame of the gene segment for influenza viral RNA polymerase subunit PB1, is a known virulence factor. Specifically, an asparagine-to-serine substitution at position 66 (N66S), which is found in 1918 pandemic viruses, is partly responsible for the high pathogenicity of this virus. In contrast, all H7N9 viruses lack the N66S mutation [20]. In addition, according to the sequence analysis, we found that almost all human H7N9 viruses encoded a full length PB1-F2 of 90 amino acids except the A/Pigeon/shanghai/S1069/2013 virus, which was truncated by a stop codon at position 26. Previous research had shown that truncation the PB1-F2 protein had no effect on viral replication in tissue culture but diminished virus pathogenicity and mortality in mice. Taking it into account, the A/Pigeon/Shanghai/S1069/2013 virus which isolated from a pigeon collecting on 2 and 3 April from a Shanghai market, may be have potentially to increase the risk to mammalian animals. It is noted that A/Pigeon/Shanghai/S1069/2013 virus also have two characteristic substitution, including 48 from R to Q and 53 from K to R.

Amino acid 627 of the PB2 protein is almost exclusively a lysine in human influenza virus isolates and a glutamic acid in avian influenza strains [14]. This residue was first identified as a determinant of host range in 1993, and was later shown to contribute to the pathogenicity and transmission in mammalian hosts in H5N1 and H7N7 subtype influenza virus infection, when the residue substituted from E to K [12] [15] [22]. Interestingly, PB2-627K is rare among avian H9N2 PB2 proteins [9]. In keeping with this finding, the avian and environment H7N9 influenza viruses encode PB2-627E. By contrast, most human H7N9 viruses encode PB2-627K. Only A/Shanghai/4/2013, A/Zhejiang/2/2013 and A/Zhejiang/DTID-ZJU01/2013 maintain the residue with PB2-627E. Different from the other H7N9 virus, the A/Zhejiang/2/2013 and A/Zhejiang/DTID-ZJU01/2013 virus PB2 have D701N instead of E627K, which can reportedly compensate for the lack of 627K in terms of increasing transmission, as well as enhancing the virus replication. Similarly to E627K, a change of PB2 amino acid 701 from aspartic acid to asparagines has been implicated in expanding the host range. The PA protein, which has function in both transcription and replication, has been reported to contribute to the regulation of the host response upon viral infection or increase the virulence of a low-pathogenic avian H5N2 influenza virus [23]-[25]. Interestingly, compared with the genome sequence, we found the characteristic mutation at position 308, except A/Hangzhou/3/2013 and A/Environment/Wuxi/1/2013 viruses, almost all viruses showed 308I, the other two viruses had 308 V. Previous study had shown that the A/Wuxi/1/2013 and A/Wuxi/2/2013 were able to transmit from person to person [26], though the limited and non-sustainable transmissibility, and demonstrated that the mutation of I308V may contribute to increasing the viral transmission.

The pathogenesis and transmissibility of influenza viruses is a polygenic trait wherein molecular determinants may differ among animal species [27]. The main mechanisms that facilitate the acquisition of virulence factors among influenza viruses are mutations due to faulty viral RNA replication and genetic reassortment with cocir-

culating influenza viruses [28] [29]. The polymerase subunits were responsible for the transcription and replication of the viral RNA genome in the nuclei of infected cells. Efficient RNA polymerase inducing high speed of virus growth can out-compete the antiviral response of the infected host cells. Notably, each of the previous 20th-century influenza virus pandemic strains has included a novel PB1 in addition the new HA and/or NA segment. Furthermore, the compatibility of the polymerase subunits may be a restricting factor for influenza virus reassortment [30]. Therefore, we should highlight the pivotal role of the viral polymerases in the evolution of influenza viruses with respect to reassortment and the production of new, more-virulent pandemic viruses (**Figure 1**, **Table 1**).

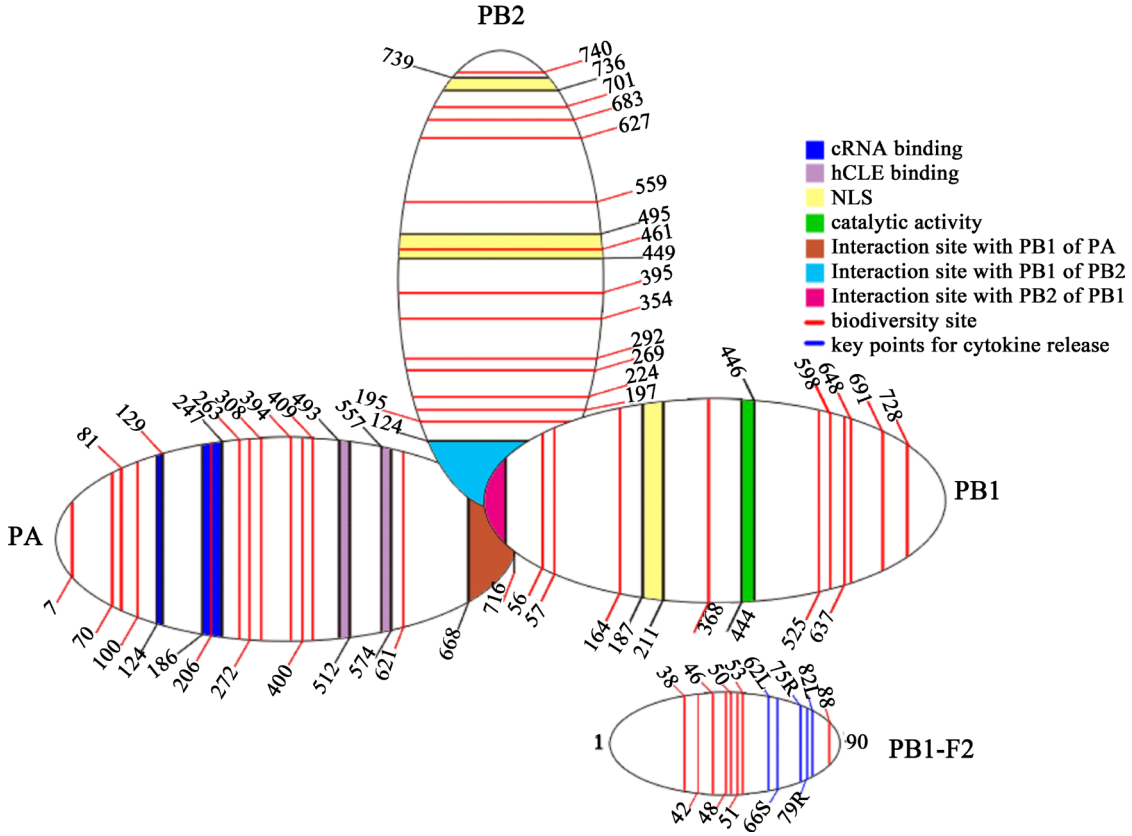

Figure 1. Diversity of the polymerase proteins of the H7N9 Influenza A Virus.

Table 1. Identification of differential amino acids of RNA polymerase in the H7N9 influenza A virus.

	PB1									
	56	57	164	368	528	598	637	648	691	728
A/Shanghai/1/2013	T	T	I	I	V	M	V	A	K	I
A/Shanghai/2/2013	T	T	I	V	V	L	I	A	K	I
A/Shanghai/3/2013	T	T	I	V	V	L	I	A	K	I
A/Shanghai/4/2013	T	T	I	V	V	L	I	A	K	I
A/Shanghai/4664T/2013	T	T	I	V	V	L	I	A	K	I
A/Chicken/shanghai/S1053/2013	T	T	I	V	V	L	I	A	K	I
A/Pigeon/shanghai/S1069/2013	A	M	I	V	I	L	I	S	K	I
A/Environment/shanghai/S1088/2013	T	T	I	V	V	L	I	A	K	I
A/Zhejiang/01/2013	T	T	I	V	V	L	I	A	K	I
A/Zhejiang/1/2013	T	T	I	V	V	L	I	A	K	I

Continued

A/Zhejiang/2/2013	T	T	I	V	V	L	I	A	K	I
A/Zhejiang/DTID-ZJU01/2013	T	T	I	V	V	L	I	A	K	I
A/Chicken/zhejiang/DTID-ZJU01/2013	T	T	I	V	V	L	I	A	K	I
A/Hangzhou/1/2013	T	T	I	V	V	L	I	A	K	I
A/Hangzhou/2/2013	T	T	I	V	V	L	I	A	K	V
A/Hangzhou/3/2013	T	T	V	V	I	L	I	A	K	I
A/Environment/Hangzhou/34/2013	T	T	I	V	V	L	I	A	K	I
A/Anhui/1/2013	T	T	I	V	V	L	I	A	K	I
A/Wuxi/1/2013	T	T	I	V	V	L	I	A	K	I
A/Wuxi/2/2013	T	T	I	V	V	L	I	A	R	I
A/Environment/Wuxi/1/2013	T	T	I	I	V	L	F	A	K	I

	PB1-F2						
	1-38	42	46	48	50	51	53
A/Shanghai/1/2013	No deletion	C	T	R	G	T	K
A/Shanghai/2/2013	No deletion	Y	T	R	G	M	K
A/Shanghai/3/2013	No deletion	Y	T	R	G	M	K
A/Shanghai/4/2013	No deletion	Y	T	R	G	M	K
A/Chicken/shanghai/S1053/2013	No deletion	Y	T	R	G	M	K
A/Pigeon/shanghai/S1069/2013	Deletion	C	T	Q	D	M	R
A/Environment/shanghai/S1088/2013	No deletion	Y	T	R	G	M	K
A/Zhejiang/01/2013	No deletion	Y	T	R	G	M	K
A/Zhejiang/1/2013	No deletion	Y	T	R	G	M	K
A/Zhejiang/2/2013	No deletion	Y	T	R	G	M	K
A/Hangzhou/1/2013	No deletion	Y	T	R	G	M	K
A/Hangzhou/2/2013	No deletion	Y	T	R	G	M	K
A/Hangzhou/3/2013	No deletion	C	M	R	D	M	K
A/Environment/Hangzhou/34/2013	No deletion	Y	T	R	G	M	K
A/Anhui/1/2013	No deletion	Y	T	R	G	M	K
A/Wuxi/1/2013	No deletion	Y	T	R	G	M	K
A/Wuxi/2/2013	No deletion	Y	T	R	G	M	K
A/Environment/Wuxi/1/2013	No deletion	C	T	R	G	T	K

	PB2											
	195	197	224	292	354	395	461	559	627	682	701	740
A/Shanghai/1/2013	D	K	T	V	I	S	V	T	K	G	D	D
A/Shanghai/2/2013	D	K	T	I	I	A	I	N	K	G	D	D
A/Shanghai/3/2013	D	K	T	V	V	A	I	N	K	G	D	D
A/Shanghai/4/2013	D	K	T	V	I	A	I	N	E	G	D	D
A/Shanghai/4664T/2013	D	K	T	V	V	A	I	N	K	G	D	D
A/Chicken/shanghai/S1053/2013	D	K	T	V	I	A	I	N	E	G	D	D
A/Pigeon/shanghai/S1069/2013	D	R	T	V	I	A	I	N	E	G	D	D
A/Environment/shanghai/S1088/2013	D	K	T	V	I	A	I	N	E	G	D	D
A/Zhejiang/01/2013	D	K	S	V	I	A	I	N	K	G	D	D

Continued

A/Zhejiang/1/2013	D	K	S	V	I	A	I	N	K	X	D	D
A/Zhejiang/2/2013	D	K	T	V	I	A	I	N	E	G	N	D
A/Zhejiang/DTID-ZJU01/2013	D	K	T	V	I	S	I	N	E	G	N	D
A/Chicken/zhejiang/DTID-ZJU01/2013	D	K	T	V	I	A	I	N	E	G	D	D
A/Hangzhou/1/2013	D	K	S	V	I	A	I	N	K	G	D	D
A/Hangzhou/2/2013	E	R	T	V	I	A	I	T	K	G	D	D
A/Hangzhou/3/2013	D	K	T	V	I	A	I	N	K	G	D	A
A/Environment/Hangzhou/34/2013	D	K	T	V	I	A	I	N	E	G	D	D
A/Anhui/1/2013	D	K	T	V	I	A	I	N	K	G	D	D
A/Wuxi/1/2013	D	K	T	V	I	A	I	N	K	G	D	D
A/Wuxi/2/2013	D	K	T	V	I	A	I	N	K	G	D	D
A/Environment/Wuxi/1/2013	D	K	T	V	I	A	I	N	E	G	D	D

	PA												
	1 - 7	70	86	100	206	263	272	308	394	400	409	621	716
A/Shanghai/1/2013	No deletion	A	M	A	E	T	N	I	N	P	N	I	R
A/Shanghai/2/2013	No deletion	A	M	A	E	T	N	I	N	P	N	I	R
A/Shanghai/3/2013	No deletion	A	M	A	E	T	N	I	N	P	N	I	R
A/Shanghai/4/2013	No deletion	A	M	A	E	T	N	I	N	P	N	I	R
A/Shanghai/4664T/2013	No deletion	A	V	A	V	T	N	I	N	P	N	S	K
A/Chicken/shanghai/S1053/2013	No deletion	A	M	A	E	T	N	I	N	P	N	I	R
A/Pigeon/shanghai/S1069/2013	No deletion	A	M	A	E	T	N	I	N	P	N	I	R
A/Environment/shanghai/S1088/2013	No deletion	A	M	A	E	T	N	I	D	P	N	I	R
A/Zhejiang/01/2013	No deletion	A	M	A	E	T	N	I	N	P	N	I	R
A/Zhejiang/1/2013	No deletion	A	M	A	E	T	N	I	N	X	N	I	R
A/Zhejiang/2/2013	No deletion	V	M	V	E	T	D	I	D	X	S	I	R
A/Zhejiang/DTID-ZJU01/2013	No deletion	V	M	V	E	T	D	I	D	P	S	I	R
A/Chicken/zhejiang/DTID-ZJU01/2013	Deletion	A	M	A	E	T	N	I	N	P	N	I	R
A/Hangzhou/1/2013	No deletion	A	M	A	E	T	N	I	N	P	N	I	R
A/Hangzhou/2/2013	No deletion	A	M	A	E	T	N	I	N	P	N	I	R
A/Hangzhou/3/2013	No deletion	A	M	A	E	A	N	V	N	P	N	I	R
A/Environment/Hangzhou/34/2013	No deletion	A	M	A	E	T	N	I	N	P	N	I	R
A/Anhui/1/2013	No deletion	A	M	A	E	T	N	I	N	P	N	I	R
A/Wuxi/1/2013	No deletion	A	M	A	E	T	N	I	N	P	N	I	R
A/Wuxi/2/2013	No deletion	A	M	A	E	T	N	I	N	P	N	I	R
A/Environment/Wuxi/1/2013	No deletion	A	M	A	E	T	N	V	N	P	N	I	R

Figure Labels: All polymerase gene sequences from H7N9 Influenza A virus strains available in the NCBI database were downloaded for alignment analysis. The amino acid mutations and sequence homology were determined and analyzed using the Meg Align H module of the DNASTAR package. Shaded boxes represent the functional domain of the polymerase protein. The numbers represent the differential amino acid site comparing

between the H7N9 viruses.

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