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Is a highly pathogenic avian influenza virus H5N1 fragment recombined in PB1 the key for the epidemic of the novel AIV H7N9 in China, 2013?

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SUMMARY

Background: A novel avian influenza A H7N9 virus that infects humans was identified in China in 2013. This study is the first to comprehensively investigate the characteristics of genomic recombination, rather than reassortment, which has been the subject of investigation in previously reported studies.
Methods: Novel avian influenza virus (AIV) H7N9 genome sequences were obtained from the NCBI Influenza Virus Sequence Database and the Global Initiative on Sharing Avian Influenza Database (GISAID) and a representative isolate was subjected to homogeneity analysis. A phylogenetic tree was constructed. Eight segments of the isolate were analyzed to identify segments with recombination events, the corresponding recombination fragments, and breakpoints. The evolutionary history of the recombined fragments was tracked by constructing phylogenetic trees of the recombination fragments.
Results: Among the eight segments of the novel AIV H7N9 analyzed, only the PB1 segment showed a marked recombination phenomenon, with 11 recombination events; these included five actual recombination events and six possible misalignment artifact recombination events. The most notable was the recombination of a 291-nucleotide (nt) fragment at the 490–780 nt site that was affiliated to a highly pathogenic avian influenza virus (HPAIV) H5N1 (A/tree sparrow/Thailand/VSMU-16-RBR/2005). The phylogenetic tree of the 291-nt recombination fragment on the PB1 segment showed that the novel AIV H7N9 had a close genetic relationship to H9N2 and H5N1.
Conclusions: The novel AIV H7N9 might have reassorted its PB1 segment from H9N2 circulating in China, and this H9N2 PB1 might have been recombined into a highly pathogenic fragment from HPAIV H5N1, which could be the reason for the high fatality rate among patients with AIV H7N9 influenza.

1. Introduction

An epidemic of human infection with a novel avian influenza virus (AIV) H7N9 first emerged in China in 2013. As of February 23, 2015, a total of 571 laboratory-confirmed cases of human infection with avian influenza A(H7N9) virus, including 212 deaths, had been reported to the World Health Organization (WHO), giving a fatality rate of 37.13%, which is much higher than the rate of <0.25% for patients with AIV H1N1 in 2009–2010. Human cases of H7N9 emerged sporadically in the winter of 2015 in China.[1–3]

The influenza virus contains eight segments of a single-stranded RNA genome with negative polarity, and is more complex than many other single-stranded unsegmented RNA viruses. Previous studies on AIV H7N9 have focused mainly on the features of reassortment among the eight segments of its genome, whereas

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potential recombination within each segment has not yet been investigated.[4,5] Identifying and excluding the recombinant segments could provide further information on the evolution of this pathogen.[6] In order to investigate the evolutionary origin of the novel H7N9 virus and the reasons for its high virulence in humans, the characteristics of recombination in each of the eight segments of this virus was explored in the present study.

2. Materials and methods

2.1. Determination of the reference isolate of the novel H7N9

Genomes of AIV H7N9 were collected from the NCBI Influenza Virus Sequence Database (http://www.ncbi.nlm.nih.gov/genomes/FLU/aboutdatabase.html) and the Global Initiative on Sharing Avian Influenza Data (GISAID) database (http://platform.gisaid.org/epi3/frontend) on May 18, 2015. Phylogenetic trees were constructed using MEGA 5.0 software (http://megasoftware.net) and homogeneous identities were calculated using Lasergene 7 software (http://www.dnastar.com); this was done to determine which isolate of H7N9 should be selected as the reference for further studies.

2.2. Searching for large-scale homogeneous sequences serving as recombination resources

Sequences sharing high pairwise identities with each segment of the genome of the reference were obtained through the Basic Local Alignment Search Tool (BLAST), and the maximum target sequences parameter was set at 1000 (consequently, 8000 sequences in total). Based on the phylogenetic tree and homogeneous identities, sequences were removed if they satisfied the following two conditions: (1) they were isolated from the same area within a period of 2 years, and (2) they showed more than 99.0% identity, since they might be the same strain obtained from different individuals. Sequences released after March 2013 were also removed, because they obviously did not conform to the logical temporal order of recombination.

2.3. Recombination analyses for segments affiliated with the novel H7N9

Homogeneous recombination events were analyzed using the recombination detection program RDP, version 4.16 (http://www.bioinf.manchester.ac.uk/recombination/programs.shtml), as reported by Martin et al. and Boni et al.[7,8]

2.4. Phylogenetic trees of recombinant fragments

To track the evolutionary history of the recombination fragments identified, phylogenetic trees consisting of recombinant fragments were constructed. Sequences corresponding to the segments that had characteristics of recombination and that were established during the period January 2003 to February 2013, regardless of their hosts and subtypes, were downloaded from the NCBI Influenza Virus Sequence Database. After alignment using ClustalW in MEGA 5.0 software, all of the segments were trimmed into the length corresponding to the identified recombination fragments. The jModelTest2 program (http://darwin.uvigo.es) was then applied to estimate the likelihood value of the model to select the best model for tree construction. Maximum likelihood phylogenetic trees were bootstrapped by 1000 replicates for significance testing.

3. Results

An early isolate A/Zhejiang/DTID-ZJU01/2013 (H7N9), which displayed very high homogeneity compared to the other H7N9 isolates established in China in 2013, was selected as the reference H7N9 for this study. Identities between them were as follows: NA, 99.3–99.9%; HA, 99.3–99.9%; M, 98.4–99%; PB1, 99.3–99.9%; NEP/NS1, 97.6–99.9%; NP, 99.2–99.7%; PA, 99.7–99.8%; and PB2, 96.7–99.8%.

After pre-processing the sequences, the numbers of sequences used for recombination analysis corresponding to each segment of the novel H7N9 were 384 PB2, 389 PB1, 423 PA, 247 HA, 414 NP, 289 NA, 411 NS, and 372 M, in accordance with the selection criteria.

Among the eight segments of the reference isolate genome, only the PB1 segment displayed notable evidence of recombination; a total of 11 recombination events were detected, including five possible recombinations and six possible misalignment artifact recombinations. The five fragments were derived from subtypes of influenza virus from different regions or host origins, with length ranging from 41 to 291 nucleotides (nt). Most notable was a 291-nt fragment recombination at the 490–780 nt site. This was affiliated with a highly pathogenic avian influenza virus (HPAIV) isolate of A/tree sparrow/Thailand/VSMU-16-RBR/2005 (H5N1) (accession number EF178509), which was responsible for a regional epidemic of highly pathogenic avian influenza in Southeast Asia in 2005 (Figure 1, Table 1).

A total of 1368 sequences from the period January 2003 to February 2013 were downloaded to track the evolutionary history of this 291-nt recombination fragment in PB1 of the novel H7N9. After evaluation of the likelihood value, GTR+G was finally chosen [AIC (Akaike information criterion) = 58 725.19] as the optimum model to construct the maximum likelihood phylogenetic tree. It was confirmed that the novel AIV H7N9 might have a close genetic relationship to the H9N2 viruses isolated from Eastern China in 2009–2012, since they were located within the same lineage on the phylogenetic tree. Another phylogenetic lineage, composed of 2007–2009 H9N2 and 2007–2010 H5N1 strains, was closely related to the aforementioned lineage. This indicates that the procedure of recombination into the PB1 might already have been accomplished around 2007; this recombinant PB1 prevailed in

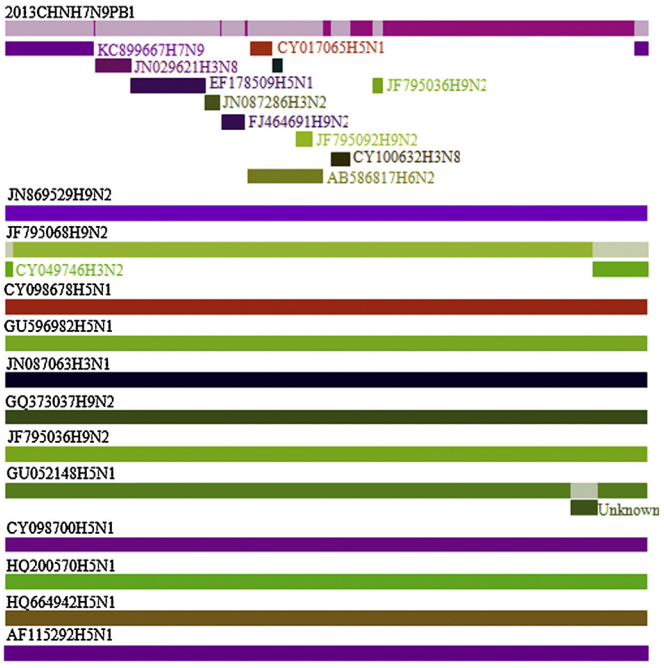

Figure 1. Patterns of recombination of the PB1 segment of the 2013 novel avian influenza virus H7N9 from China (partial segment). Series of recombinant fragments within the PB1 segment of avian influenza virus H7N9 isolated in China in 2013 are displayed.