

Genome Sequence of a Natural Reassortant H5N2 Avian Influenza Virus from Domestic Mallard Ducks in Eastern China

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Here, we report the genomic sequence of a Chinese reassortant H5N2 avian influenza virus which possessed the polybasic motif PLREKRRK-R/GL at the hemagglutinin cleavage site. Phylogenetic analysis showed that all eight genes were of the Eurasian lineage, five of which were highly homologous to the endemic clade 2.3.4 H5N1 viruses and their H5N5 reassortant descendants. These data suggested that novel multisubtypic NA reassortants bearing the H5N1 backbone could be generated through genetic reassortment in H5N1 circulating regions, which will help in understanding the evolution and segment reassortment mechanism of H5 subtype avian influenza viruses.

Avian influenza virus, a member of the family *Orthomyxoviridae*, is specified into 16 HA (hemagglutinin) subtypes and 9 NA (neuraminidase) subtypes according to the antigenicity of the two surface glycoproteins HA and NA (7, 8). Among these, the H5N1 subtype combination of the Gs/GD lineage is of great concern, as it has caused disastrous damage to the poultry industry and has posed a serious threat to public health (7). In particular, the clade 2.3.4 Fujian-like H5N1 viruses have gradually become endemic in China since 2005 (3, 5) and continue to evolve actively in China, Vietnam, Laos, and neighboring regions (9). Furthermore, they could even provide the backbones to generate a novel NA subtype of H5 highly pathogenic avian influenza (HPAI) viruses, such as H5N5, via genetic reassortment (1).

In this study, an H5N2 virus, A/duck/Jiangsu/m234/2012 (m234), was isolated from apparently healthy domestic mallard ducks in the Jiangsu Province of eastern China in January 2012. To characterize this strain in detail, we determined the whole genome sequence by reverse transcription-PCR performed with the universal primer set (2) and by sequencing using the ABI 3730 DNA analyzer (Applied Biosystems).

Eight negative-sense RNA segments, including PB2, PB1, PA, HA, NP, NA, M, and NS genes, constitute the viral genome, with full lengths of 2,341, 2,341, 2,233, 1,776, 1,565, 1,466, 1,027, and 890 nucleotides, respectively. In contrast to the recently reported Chinese H5N2 isolates (4, 10), m234 carried the polybasic motif PLREKRRK-R/GL at the HA cleavage site, which was consistent with the molecular signature of HPAI virus. However, typical residues Q226 and G228 (H3 numbering) in the receptor binding pocket of HA protein indicated that m234 retained the avian-like SA α 2,3Gal receptor preference. The 5-amino-acid deletion at positions 80 to 84 of the NS1 protein, which has been proven to exist in more and more H5N1 viruses isolated from 2000 onwards (6), was not detected in m234.

Phylogenetic analysis showed that all eight genes were of the Eurasian lineage. The PB2, PA, HA, NP, and M genes were highly homologous (97% to 98%) to the previously identified reassortant HPAI H5N5 viruses A/duck/Eastern China/031/2009 and A/duck/Eastern China/008/2008 (1) and their clade 2.3.4 H5N1 progenitors A/duck/Eastern China/108/2008 and A/duck/Eastern China/909/2009. The PB1 and NA genes shared the greatest DNA sequence identities (over 98%) with A/duck/Jiangxi/k0701/

2009(H11N2), whereas the NS gene was most closely related (over 95%) to A/duck/Nanchang/1904/1992(H7N1), which was isolated 2 decades ago. Therefore, we speculated that m234 may probably be reassorted from the above-mentioned duck origin avian influenza viruses. The isolation of this natural reassortant H5N2 virus further highlights that the genetic stability between the HA and NA subtype pairing of the clade 2.3.4 H5N1 HPAI viruses may have been disequibrated to generate novel multisubtypic NA reassortants based on their genetic backbones.

In conclusion, the genome information of A/duck/Jiangsu/m234/2012(H5N2) will be helpful to study the evolution and segment reassortment mechanism of H5 subtype avian influenza virus in areas where the virus is endemic.

Nucleotide sequence accession numbers. The genome sequences of A/duck/Jiangsu/m234/2012(H5N2) were deposited in GenBank under accession numbers [JX507352](#) to [JX507359](#).

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