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Molecular phylogeny and evolutionary dynamics of matrix gene of avian influenza viruses in China

Peng Xiaorong, Wu Haibo, Peng Xiuming, Jin Changzhong, Lu Xiangyun, Xie Tiansheng, Cheng Linfang, Liu Fumin, Wu Nanping *

State Key Laboratory for Diagnosis and Treatment of Infectious Diseases, Collaborative Innovation Center for Diagnosis and Treatment of Infectious Diseases, The First Affiliated Hospital, School of Medicine, Zhejiang University, 310003 Hangzhou, China

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ABSTRACT

In China, several subtype avian influenza viruses consistently circulate in poultry. Numerous studies have focused on the evolution of the hemagglutinin gene; however, studies on the evolution of the matrix (M) gene are limited. In this study, a large-scale phylogenetic analysis of M gene sequences of avian influenza viruses isolated in China revealed that the M gene has evolved into six different lineages denoted as I–VI. The majority of lineages I and IV were isolated in terrestrial birds, while the majority of lineages II, III, V and VI were isolated in aquatic birds. Lineage I included 148 H9N2 subtype viruses (72.2%), lineage II comprised of 63 H6 subtype viruses (100%), and lineage IV included 157 H5 subtype viruses (97.5%). The mean substitution rates of different lineages ranged from 1.32×10^{-3} (lineage III) to 3.64×10^{-3} (lineage IV) substitutions per site per year. According to the most recent common ancestor of all lineages, lineage III was the oldest lineage, formed in 1981 or even earlier. And lineage V was the most recent, established around the year 2000. Selective pressure on M2 was stronger than that on M1. The strongest selection pressure was observed in lineage IV. In addition, site-by-site analyses identified 8 positive selection sites, all in M2. Most of the sites (5 out of 8) were located in the extracellular domain, which is an antigen for vaccine development. The positive selection sites (amino acid positions 66, 82 and 97) are likely associated with virus budding. This study enhanced our knowledge of M gene evolution of avian influenza viruses, and is expected to improve the early detection of new viruses and lead to vaccine development.

1. Introduction

In China, several subtype avian influenza viruses (AIVs) consistently circulate in poultry, including highly pathogenic avian influenza (HPAI, i.e. H5) (Ma et al., 2015), and low pathogenic avian influenza (i.e. H9 and H6) (Huang et al., 2012). Through gene reassortment and antigenic drift, many novel subtype AIVs have emerged (i.e. H7N9, H10N8 and H5N8) (Chen et al., 2014; Chen et al., 2013; Wu et al., 2014). These AIVs not only kill thousands of poultry, causing huge economic losses, but also represent a threat to humans. Thus, it is necessary to understand the evolutionary processes of AIV to gain a better knowledge of this pandemic virus. Numerous studies have focused on the evolution of the hemagglutinin antigen (HA) gene (Huang et al., 2012; Ma et al., 2015; Wu et al., 2010); however, only a few studies have addressed the evolution of the matrix (M) gene (Chander et al., 2013).

The M gene encodes two partly overlapping proteins, a highly conserved 252-amino-acid M1 protein and a 97-amino-acid M2 protein (Scholtissek et al., 2002; Sun et al., 2010). The M1 protein binds to the cytoplasmic tails of HA and neuraminidase (NA), and bridges interactions between the viral lipid membrane and the ribonucleoprotein (RNP) core (Schmitt and Lamb, 2005). M1 and M2 play a vital role in viral assembly and budding. During viral assembly, M1 recruits several viral components (HA, NA, M2 and RNP) to the site of assembly (Gomez-Puertas et al., 2000). M2 initially stabilizes the site of budding, and subsequently alters membrane curvature, causing membrane scission and the release of the progeny virion (McCown and Pekosz, 2006; Rossman and Lamb, 2011).

The M2 protein is a transmembrane protein: position 1–24 is the extracellular domain, position 25–43 is the transmembrane domain and position 44–97 is the cytoplasmic domain (Scholtissek et al., 2002). The transmembrane domain of M2 has

* Corresponding author.
E-mail addresses: 699xiaorong@163.com (P. Xiaorong), flwnp2013@163.com (W. Nanping).

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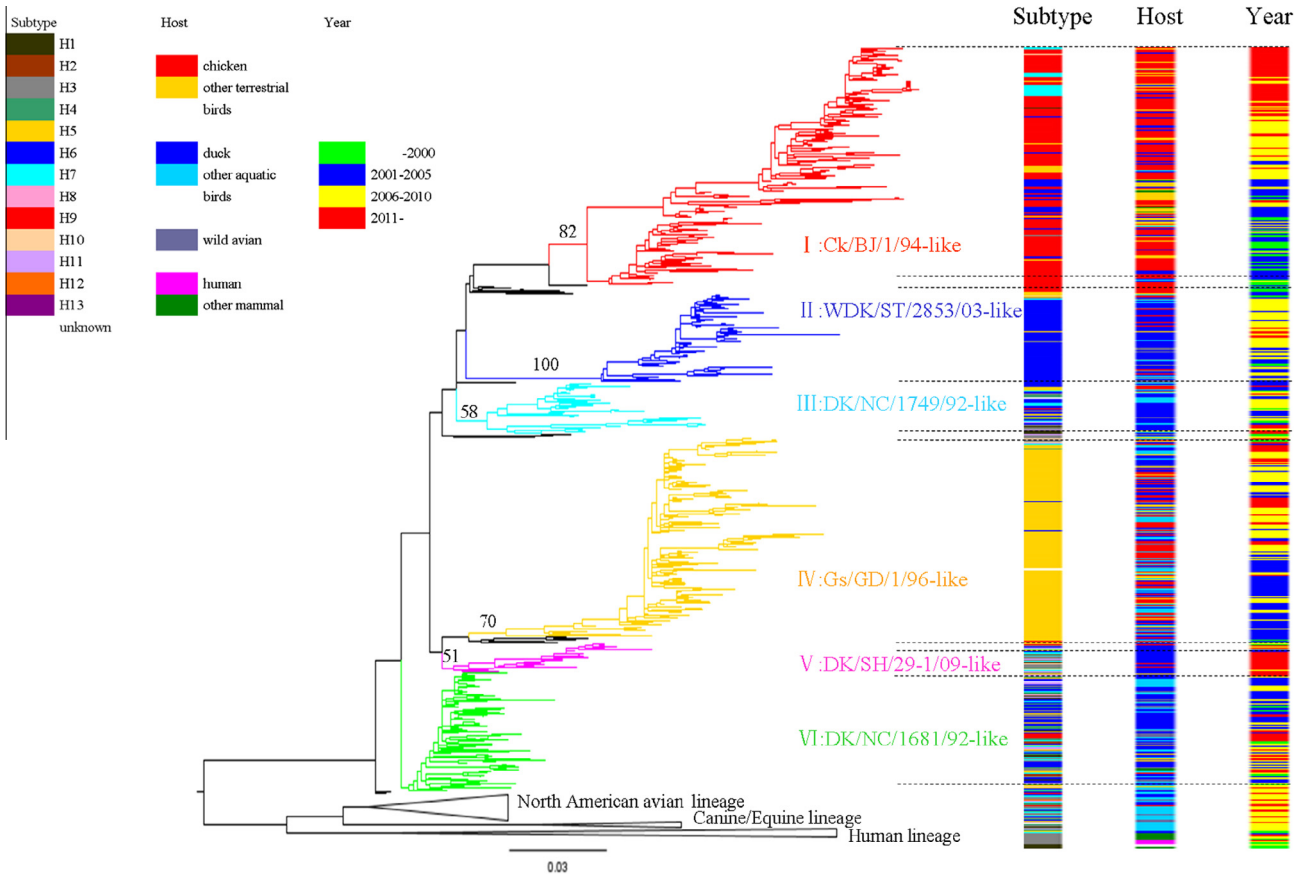

Fig. 1. Phylogenetic trees for the M gene. The phylogenetic trees were constructed using the maximum likelihood method. Scale bar shows the evolutionary distance. The subtype, host and year of each M gene are indicated by different colored bars (see legend). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

ion channel activity, which is the target of adamantanes, the first class of antiviral drugs approved for treatment of human influenza (Salter et al., 2011). The widespread use of adamantanes against influenza has led to the emergence of resistant virus strains (Bright et al., 2005). Adamantine resistance is characterized by a mutation in one of five sites (positions 26, 27, 28, 31 or 34) in the M2 gene, although the most commonly observed mutation is S31N (Wang et al., 2013).

The extracellular domain of the M2 protein (M2e), which contains 24 residues at the N-terminus, is highly conserved in all human epidemic strains, independent of subtype (Liu et al., 2005). Therefore, M2e serves as an attractive target for the development of universal influenza subunit vaccines (Fiers et al., 2004). Nevertheless, an avian-type M2e consensus amino acid sequence is up to 5 positions different from a human-type consensus sequence (Liu et al., 2005). Whether the M2e of AIVs in China is conserved or is under positive selection is unknown. Therefore, understanding of evolution of the M gene is very important and is practically relevant.

In this study, a large-scale phylogenetic analysis of M genes of AIVs isolated in China was conducted to infer their evolutionary relationship. The substitution rate and the most recent common ancestor (TMRCA) were estimated using a Bayesian Markov Chain Monte Carlo (MCMC) method. We also estimated non-synonymous to synonymous substitution rate ratios (dN/dS ratio), and investigated the positive selection sites for each lineage. These analyses increase our knowledge of M gene evolution in AIVs, and are expected to improve AIVs surveillance and vaccine development.

2. Materials and methods

2.1. Sequence data

The nucleotide sequences of M gene were obtained from GenBank, hosted by the National Center for Biotechnology Information, on December 31, 2014 (Bao et al., 2008). All sequence data for the strains with a full-length M gene of any subtypes of AIVs isolated in China were included. Identical sequences in a dataset were represented by the oldest sequence in the group. Sequencing data were obtained together with information about the host, subtype, isolation year and isolation place. The sequences were highly similar; therefore, we picked at least one sequence per year, per subtype, per place for further study. A total of 644 sequences were obtained (the accession numbers are listed in additional file 1). All segments were aligned using the default settings in MUSCLE v3.5 (Edgar, 2004).

2.2. Phylogenetic tree analysis

To enhance the phylogenetic analysis of M gene of AIVs in China, we also included several M genes of AIVs from several geographical origins (North-American and other Eurasian out of China) and different host origins (human, swine, canine and equine). The sequence data for the coding regions only were used; i.e. from nucleotide position 26 to 1007. Phylogenetic analysis was conducted using the maximum likelihood (ML) method in RAxML (Stamatakis, 2014). Analyses of 1000 bootstrap replicates were performed using GTR-GAMMA, the GTR model of nucleotide

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