Characterization of Low Pathogenic H5 Subtype Influenza Viruses From Eurasia: Implications for the Origin of Highly Pathogenic H5N1 Viruses

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Highly pathogenic avian influenza (HPAI) H5N1 viruses are now endemic in many Asian countries. The immediate precursor of these HPAI viruses was recognized as A/Goose/Guangdong/1/96 (Gs/Gd)-like H5N1 HPAI viruses first detected in Guangdong in 1996. However, precursors of the Gs/GD-like viruses and their subsequent reassortants have not been fully determined. Here we characterize low pathogenic avian influenza (LPAI) H5 subtype viruses isolated from poultry and migratory birds in southern China and Europe from the 1970s to the 2000s. Phylogenetic analyses revealed that Gs/GD-like virus was likely derived from an LPAI H5 virus in migratory birds. However, its variants arose from multiple reassortments between Gs/GD-like virus and viruses from migratory birds, or with those Eurasian viruses isolated in the 1970s. It is of note that unlike HPAI H5N1 viruses, those recent LPAI H5 viruses have not become established in aquatic or terrestrial poultry. Phylogenetic analyses revealed the dynamic nature of the influenza gene pool in Eurasia with repeated transmissions between the eastern and western extremities of the continent. The data also shows reassortment between influenza viruses from domestic and migratory birds in this region that has contributed to the expanded diversity of the influenza gene pool among poultry in Eurasia.

Introduction

Aquatic birds are considered the natural reservoirs of influenza A virus as all known subtypes (H1-H16 and N1-N9) have been isolated from them [1]. Genetic analyses have also suggested that all influenza viruses found in other hosts were derived from those viruses resident in aquatic birds [2]. Influenza viruses from aquatic birds have occasionally transmitted to terrestrial poultry leading to disease outbreaks, however, only some H5 and H7 subtypes influenza viruses have evolved into highly pathogenic strains that have caused significant mortality in poultry. Previous studies have demonstrated that, in the case of H5 and H7 subtypes, low pathogenic avian influenza viruses (LPAI) precursors may evolve into HPAI once transmitted to domestic poultry [3-5].

Results

Prevalence of low pathogenic H5 influenza viruses. Between July 2000 and December 2005, a total of 167,858 samples of domestic duck, geese and migratory ducks were collected in southern China and Hong Kong. Seventy-two LPAI H5 viruses were isolated on 10 sampling occasions during over-winter season (isolation rate 0.043%). The main body of those isolates was from sentry farm ducks or directly from migratory birds. Only two isolates were detected from retail market bird. In 1970s' surveillance, among 11,798 market samples 23 LPAI H5 viruses were isolated on 10 sampling occasions (isolation rate 0.2%). Most of the viruses were from domestic ducks with only one isolated from goose. It must be noted that no LPAI H5 viruses were recognized in terrestrial poultry in our surveillance.

Phylogenetic analysis of the surface genes. Phylogenetic analysis of the H5 HA gene showed that all viruses separated into the American and Eurasian lineages (Figure 1A). Within the Eurasian lineage two major sublineages of "early" and "contemporary" viruses are apparent. The first sublineage consists predominantly of older viruses from across the continent, including those viruses isolated in Hong Kong from 1976 to 1980, along with viruses from Germany, Japan and the United Kingdom. There is only one LPAI H5 virus from Italy (Ck/Italy/9097/97) that falls into this group which was introduced recently to chicken from this gene pool. Therefore, this sublineage of H5 subtype viruses probably represents the early domestic Eurasian gene pool of H5 viruses (Figure 1A). The second major Eurasian sublineage is composed of three distinct groups. Group 1 contains all but one of those recent viruses from China and Italy characterized in present study, along with other Eurasian isolates, from both domestic and migratory birds. However, four Italian viruses isolated during the outbreaks from 1997 to 1998 and one obtained from a mallard clustered together within this group, suggesting that after introduction into terrestrial poultry those viruses had undergone significant evolution. The phylogeny of this group, together with that of the "early" sublineage, indicates the continued genetic exchange within this influenza gene pool between the eastern and western extremities of the Eurasian continent. Group 2 consists exclusively of viruses from Western Europe, with the exception of the virus Dk/Chany Lake/9/03 that was isolated in Novosibirsk, Russia in Central Asia (Figure 1A). In contrast, group 3 contained viruses from the Western Pacific isolated from domestic duck and swans, along with HPAI Gs/GD-like H5N1 viruses that have subsequently spread throughout Eurasia [6]. This group therefore seems to match one of the major migratory flyways in this region. Phylogenetic analysis showed that three N2 genes from recent southern China H5N2 isolates (Gs/GY/3799/05, Dk/JX/1286/05 and Dk/JX/3345/05) were closely related to the N2 genes of viruses obtained from mallard in Italy and duck in Hokkaido, Japan (data not shown). Analysis also showed that the N3 gene from those recent H5N3 viruses isolated in southern China are closely related to H7N3 subtype viruses from domestic and migratory birds in Italy from 2001 to 2003 (data not shown). Therefore, both the HA and NA genes of those H5N2 and H5N3 viruses recently isolated from domestic ducks or migratory birds in southern China were from the Eurasian lineage and appear to be derived from viruses resident in migratory birds. Phylogenetic analysis of N1 NA also revealed that the NA gene of Gs/GD-like virus was most closely related to Dk/Hokkaido/55/95 (H1N1). Another H5N1 variant (Ck/Hebei/718/01) had an N1 that was almost identical to African Starling/England-Q/983/79 (H7N1), which was investigated as a vaccine candidate in China [7].

Phylogenetic analysis of the internal genes. In general, all internal gene trees, represented by the M gene, were divided into the American and Eurasian lineages, and the "early" and "contemporary" sublineages were observed within the Eurasian lineage (Figure 1B). The internal gene phylogenies further highlight the dynamic nature of the influenza gene pool in Eurasia with viruses from the east and west frequently clustering together. For the LPAI H5N2 virus, Ck/Ibaraki/1/05 all available gene segments clustered with the Mexico-like HPAI from 1994. It is noteworthy that several Gs/GD-like variants isolated from northern China from 1997 to 2004 consistently clustered within the "early" Eurasian sublineage (Figure 1B). Most of the LPAI H5 viruses from southern China clustered or grouped together with some viruses from European viruses, including those Italian HPAI and LPAI viruses. To identify the possible source of Gs/GD-like HPAI H5N1 viruses and its variants, we summarized their most phylogenetically closely related viruses for each of the gene segments (Table 1). For Gs/GD-like viruses, three of the gene segments were likely derived from migratory birds, and four of the genes are most closely related to those viruses isolated from ducks in Hokkaido, Japan. Furthermore, some of the variants isolated from our surveillance during 2001 to 2005 in southern China contain gene segments that are most closely related to gene segments from viruses in the migratory bird gene pool (Table 1). It is noteworthy that these analyses confirm that several H5N1 variants isolated from northern China from 1997 to 2004 contained many gene segments belonging to the early Eurasian gene pool identified from viruses isolated in the 1970s and 1980s. Moreover, it is surprising that one H5N1 virus (Ck/Hebei/718/01) contained an NA N1 gene with very high homology (98.3%) to that of African starling/England-Q/983/79 (H7N1).

Discussion

The findings of present study revealed that LPAI H5 viruses were isolated predominantly from migratory or sentry ducks during the winter, and were barely detected in market waterfowl and not found in terrestrial poultry in southern China from 2000 to 2005. This suggests that interspecies transmission of LPAI H5 virus from migratory birds to domestic waterfowl did occur, but that those viruses did not subsequently become prevalent in aquatic or terrestrial domestic birds. In contrast, influenza surveillance in the 1970s revealed that LPAI H5 viruses were found year-round in domestic waterfowl, indicating that there has been a change in the ecology of influenza in southern China. This likely resulted from the long-term endemicity of HPAI H5N1 viruses and extensive vaccination in poultry leading to increased host selection pressure. In the present study, the phylogenies of Gs/GD-like H5N1 virus genes either clustered with viruses from migratory birds or with those viruses isolated from ducks in Hokkaido, Japan (Table 1). As no systematic surveillance was conducted from 1980 to 1997 in southern China, we failed to identify the direct precursor of Gs/GD-like virus which emerged in 1996 [8]. Even though it is not clear whether those Hokkaido viruses were from domestic or migratory ducks, many of them clustered directly with the viruses isolated from migratory ducks at Poyang Lake, Jiangxi and Mai Po Marshes, Hong Kong since 2002. We therefore speculate that the duck viruses from Hokkaido may also be of migratory bird derivation. Thus, the available findings suggest that Gs/GD-like H5N1 virus may be derived from a LPAI H5N1 virus in migratory waterfowl along the West Pacific migratory flyway. A precedent for such an introduction and subsequent change from LPAI to HP has also been observed in Europe [3-5].

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Figure 1. Phylogenetic relationships of the HA (A) and M (B) genes of representative influenza A viruses isolated in Asia, American and Europe. Numbers above below the branch nodes indicate neighbor-joining bootstrap values >50% and Bayesian posterior probabilities >95, respectively, analyses were based on nucleotides 22-1032 of the HA gene and 26-947 of the M gene. The HA and M gene trees were rooted to A/Japan/305/57 and A/Equine/Prague/1/56, respectively. Scale bar, 0.1 nucleotide substitutions per site.

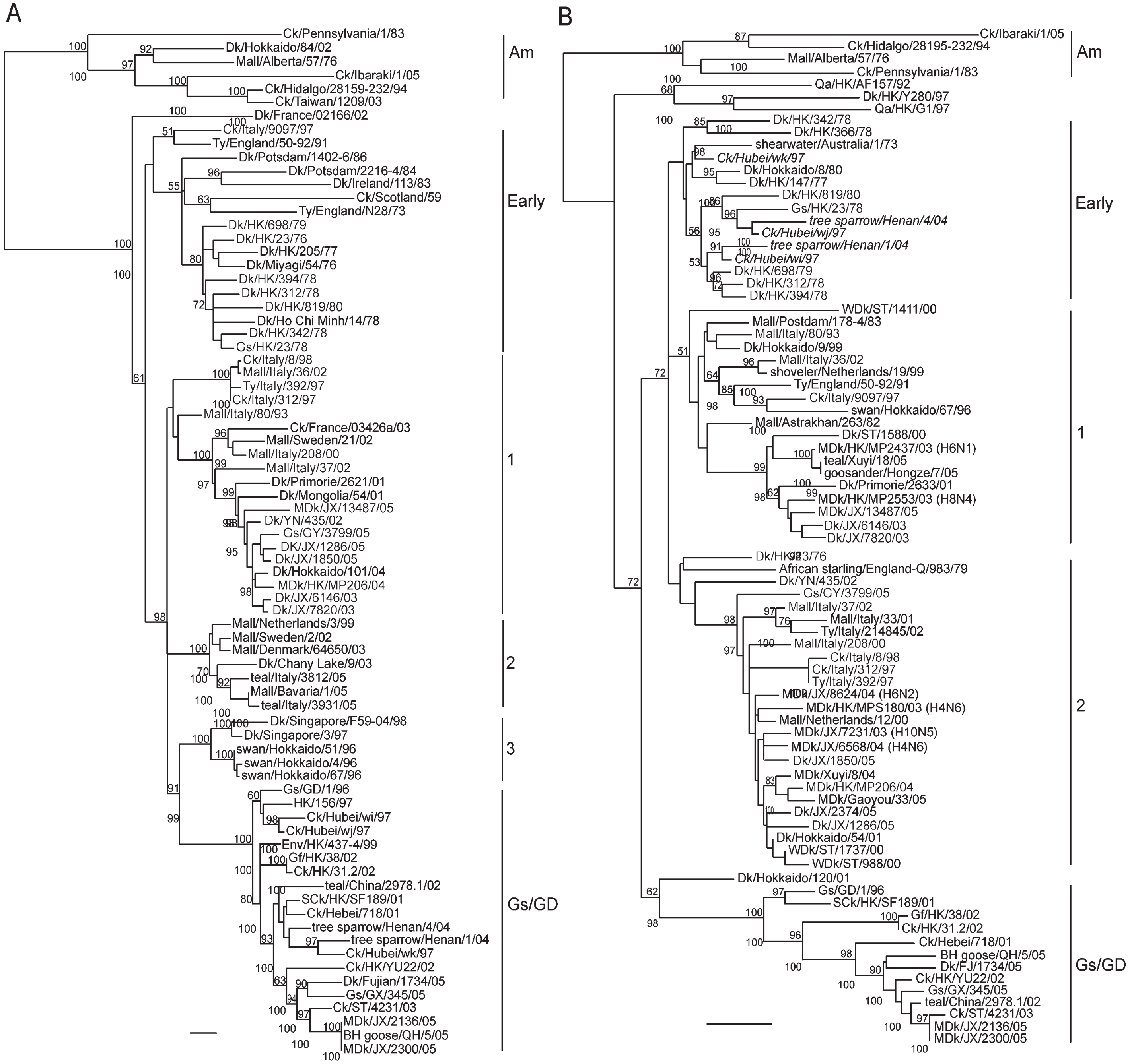

Table 1. Possible sources of gene segments of highly pathogenic H5N1 influenza viruses isolated in China, 1996 to 2005.

Virus	Gene segment[a]							
	PB2	PB1	PA	HA	NP	NA	M	NS
Gs/GD/1/96	MB?	Hokkaido	MB?	Hokkaido	unknown	Hokkaido	Hokkaido	MB
SCk/HK/SF189/01	MB?	Gs/GD	Gs/GD	Gs/GD	Gs/GD	Gs/GD	Gs/GD	unknown
Gf/HK/38/02	H9N2	MB	Early	Gs/GD	unknown	Gs/GD	Gs/GD	MB
Ck/HK/31.2/02	MB	MB	Early	Gs/GD	MB?	Gs/GD	Gs/GD	MB
teal/China/2978.1/02	Gs/GD	Gs/GD	MB	Gs/GD	MB	Gs/GD	Gs/GD	unknown
Ck/HK/YU22/02	MB	Gs/GD	MB	Gs/GD	unknown	Gs/GD	Gs/GD	unknown
Ck/ST/4231/03	MB	Gs/GD	MB	Gs/GD	unknown	Gs/GD	Gs/GD	unknown
Gs/GX/345/05	Gs/GD	Gs/GD	MB	Gs/GD	unknown	Gs/GD	Gs/GD	unknown
Dk/FJ/1734/05	MB?	Gs/GD	MB	Gs/GD	unknown	Gs/GD	Gs/GD	unknown
Ck/Hubei/wi/97	Early	–	Early	Gs/GD	Early	Gs/GD	Early	Early
Ck/Hubei/wj/97	Early	Early	Early	Gs/GD	Early	Gs/GD	Early	Early
Ck/Hubei/wk/97	unknown	Early	Early	Gs/GD	Early	Gs/GD	Early	Early
Ck/Hebei/718/01	–	Gs/GD	MB	Gs/GD	Early	**AS/Eng**	Gs/GD	Gs/GD
TS/Henan/1/04	**Early**	**Early**	**Early**	**Gs/GD**	**Early**	**Gs/GD**	**Early**	**Early**
HK/156/97	G1	G1	G1	Gs/GD	G1	W312	G1	G1

AS/Eng, African starling/England-Q/983/79; Early, 1970s Eurasia aquatic virus; Unknown, cannot identify source from the currently available influenza data; –, gene sequence not available; G1, Qa/HK/G1/97-like (H9N2) virus; W312, teal/HK/W312/97-like (H6N1) virus; Hokkaido, virus sequences published with the names Dk/Hokkaido/9/99 (PB1), swan/Hokkaido/4/96 (HA), Dk/Hokkaido/55/96 (NA), Dk/Hokkaido/120/01 (M).