Epidemiologic Study of Human Influenza Virus Infection in South Korea from 1999 to 2007: Origin and Evolution of A/Fujian/411/2002-Like Strains^{∇‡}

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Influenza epidemics arise through the accumulation of viral genetic changes, culminating in a novel antigenic type that is able to escape host immunity. Following an outbreak of the A/Fujian/411/2002-like strains in Asia, including China, Japan, and South Korea, in 2002, Australia and New Zealand experienced substantial outbreaks of the same strains in 2003, and subsequently worldwide outbreaks occurred in the 2003-2004 season. The emergence of A/Fujian/411/2002-like strains coincided with a higher level of influenza-like illness in South Korea than what is seen at the peak of a normal season, and there was at least a year's difference between South Korea and the United States. Genetic evolution of human influenza A/H3N2 viruses was monitored by sequence analysis of hemagglutinin (HA) genes collected in Asia, including 269 (164 new) HA genes isolated in South Korea from 1999 to 2007. The Fujian-like influenza strains were disseminated with rapid sequence variation across the antigenic sites of the HA1 domain, which sharply distinguished between the A/Moscow/10/1999-like and A/Fujian/411/2002-like strains. This fast variation, equivalent to approximately 10 amino acid changes within a year, occurred in Asia and would be the main cause of the disappearance of the reassortants, although the reassortant and nonreassortant Fujian-like strains circulated simultaneously in Asia.

Influenza is an important respiratory infectious disease causing seasonal epidemics or occasional pandemics across the world, with considerable morbidity and mortality. The influenza outbreaks are associated with antigenic variation of influenza viruses. Annual influenza epidemics typically occur during the winter season in temperate regions, whereas tropical regions may function as permanent mixing pools for viruses, providing a ready source of extended viral transmission (7, 26). Antigenic and genetic analyses revealed that there was a continuous circulation of human influenza A/H3N2 viruses in East and Southeast Asia via a regional network from which epidemics in the temperate regions were seeded (22). In particular, southern China was considered a potential epicenter for the emergence of novel influenza virus strains (23).

The enveloped influenza virus contains eight segments of negative-sense single-stranded RNA, each of which codes for a particular viral protein(s). The gene segment coding for a surface glycoprotein hemagglutinin (HA) is of major importance because HA is the primary target of immune response and the

primary component of influenza vaccine. HA is a homotrimeric protein synthesized as a single polypeptide, HA0, that is cleaved into two subunits, HA1 and HA2, for receptor binding and cell entry (18). Antibodies against HA are elicited during virus infection to inhibit binding with receptor effectively (27). Accumulation of amino acid variation in HA is clustered in variable antigenic sites around the receptor binding site, which leads to gradual antigenic drift in the influenza viruses. It was previously proposed that an antigenic drift variant of epidemiological importance usually requires changes of at least four amino acids across two or more antigenic sites, but a single amino acid substitution at one antigenic site can cause sufficient antigenic change (9, 10). The influenza virus can also acquire a new subtype by reassortment of one or more gene segments, which, combined with antigenic drift, provides the basis for the remarkable antigenic variability in viral populations (12).

A/Moscow/10/1999-like or antigenically equivalent A/Panama/2007/1999-like strains of H3N2 have been circulating worldwide since 1999. The emergence of A/Fujian/411/2002-like strains caused an epidemic in China, Japan, and South Korea in 2002 (3, 13, 21). It was shown that a two-amino-acid substitution was critical for antigenicity distinct from that of A/Panama/2007/1999 (14). Interestingly, a descendant of the Fujian strain reassorted, which caused an unusually severe influenza season in Australia and New Zealand in 2003 and in North America and Europe and worldwide in the 2003-2004 season (2, 4, 13). This reassortment caused a minor clade to provide a HA gene that later became part of the dominant strain in the same season (4, 13), reaching South America after an additional 6 to 9 months (22). The appearance of the Fujian strains thus

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prompted a change in the selection of vaccine components in 2004. However, nonreassortant strains were the dominant strains in Asia in the 2002-2003 season and thereafter.

The elucidation of how and when a new influenza virus emerges as an epidemic strain requires a deeper understanding of the mechanisms that underlie viral evolution. We have determined the nucleotide sequence of the HA gene segments of influenza viruses in nasal swabs collected from infected patients aged 6 months and older during the 1999-2007 influenza seasons in South Korea. At the same time, influenza-like illness was monitored during each season, and the phylogeny of HA sequences available worldwide was analyzed to investigate the origin and evolution of the H3N2 Fujian strains.

MATERIALS AND METHODS

Virus collection and isolation. Nasopharyngeal swabs were obtained from outpatients with symptoms of influenza-like illness (ILI), residing in Seoul and other cities in South Korea from 1999 to 2007. The samples in viral transport medium were transported to the Influenza Virus Disease Team at the Korea National Institute of Health (KNIH) in Seoul on the day of collection. One hundred-microliter aliquots of the supernatants of the nasopharyngeal swabs were inoculated onto Madin-Darby canine kidney (MDCK) cells in 48-well multiple plates which were prepared at 37°C with 5% CO₂. Virus growth was monitored at 34°C, with reference to cytopathic effects. The viruses were passaged three times to obtain sufficient virus titers for virus identification. All isolates were typed and subtyped by the hemagglutination inhibition assay (15).

RNA extraction and PCR. One hundred-microliter aliquots of the supernatant after the third culture passage were used for viral RNA extraction with an Extragen II kit (Kainos, Tokyo, Japan), according to the manufacturer's instructions. RNA was transcribed to cDNA with the influenza A virus universal primer, and the HA gene of H3N2 viruses was amplified with segment-specific primers (15).

Nucleotide sequencing and sequence analysis. The PCR products were purified with a MicroSpin S-300 HR column (GE Healthcare, Uppsala, Sweden), labeled by using a BigDye Terminator version 3.1 cycle sequencing kit (Applied Biosystems, Foster, CA), according to the manufacturer's instructions, and analyzed on an ABI 3100 automatic DNA sequencer.

Sequence alignment of the HA1 domain was performed using the MUSCLE program (8). The maximum parsimony tree of South Korean strains was inferred using the MEGA 4.0 program with a close-neighbor-interchange algorithm (25). In this analysis, 116 amino acid sequences of South Korean strains, representatives of 269 strains after excluding 153 strains that have identical amino acid sequences, were included for the reconstruction of a phylogenetic tree. A/Beijing/32/1992 was used for rooting the tree, and 7 other vaccine strains were included as reference strains for each year's epidemics. In addition, 6 strains isolated in 2009 were combined to the data set to determine the ancestral lineage of recent H3N2 strains. HA and neuraminidase (NA) maximum likelihood (ML) trees were determined to identify Fujian reassortants in Asia during two consecutive seasons from 2002 to 2004. A total of 30 New York strains, including 11 reassortant strains shown in Fig. 1 of reference 19, were incorporated to assess the phylogenetic positions of reassortants. The node support was calculated by the approximate likelihood ratio test (aLRT) using PhyML (1, 11). To examine the evolution of A/Fujian/411/2002-like strains, 521 HA1 nucleotide sequences from the National Center for Biotechnology Informatics (NCBI) GenBank and newly sequenced South Korean isolates were subjected to ML analysis. The data set for ML phylogenetic analysis contained HA1 of H3N2 viruses collected globally from 2001 to 2002, except for the sequences of less than 950 bp or ambiguous sequences. Two South Korean strains that diverged prior to A/Sydney/5/1997, i.e., A/Gyeongbuk/2/2002 and A/Gyeongbuk/304/2002, were excluded. After A/Moscow/10/1999 was used as the phylogenetic root and 2 vaccine strains that were isolated in 2004 and used as representatives of the following season's dominant strains were combined, we reanalyzed 53 strains from the ML tree to pinpoint the evolutionary pathway of the Fujian/411 origin. All ML analyses were performed using PhyML software with a GTR+I+ Γ_4 model (11).

Nucleotide sequence accession numbers. A total of 164 nucleotide sequences for the H3N2 subtype from KNIH were deposited in NCBI GenBank, (accession no. CY054107 to CY054270), and 105 H3N2 nucleotide sequences of South Korean isolates were retrieved from the NCBI Influenza Virus Resource, which are listed in Table S1 in the supplemental material.

RESULTS

Prevalence of influenza in South Korea from 1999 to 2007.

The relative prevalence of influenza virus subtypes varied from season to season. Human influenza A/H3N2 viruses were the dominant circulating strain in South Korea during the seasons from 1999 to 2007 (Fig. 1A). In addition, the 2001-2002 and 2005-2006 seasons were marked by the spread of influenza A/H1N1 viruses in peak winter weeks and B viruses in the following spring. Our data showed that H3N2 has become widespread and cocirculated with H1N1 and B viruses during the 2000-2007 seasons.

A/Moscow/10/1999-like strains were dominant in the seasons from 1999 to 2001 (Fig. 2A). The change of dominant strains in H3N2 from A/Moscow/10/1999 to A/Fujian/411/2002 caused a big epidemic worldwide in the 2003-2004 season (3, 4, 17, 21, 22). However, the influenza activity of the A/Fujian/411/2002-like viruses was already observed in China and South Korea in 2002 (6). Our epidemiologic study showed that the emergence of A/Fujian/411/2002 coincided with a higher level of influenza-like illness in South Korea than what is typically seen at the peak of a normal season. It was particularly notable that the monitoring of ILI during the 2002-2004 seasons demonstrated a 1-year difference between the ILI patient data of South Korea with those of United States (Fig. 1B). Further, the composition of influenza subtypes that circulated in the United States essentially resembled that in South Korea in each previous year (Fig. 1C).

A/H3N2 influenza viruses. HA genes of 269 isolates of human influenza A/H3N2 viruses that had been collected and sequenced in South Korea from 1999 to 2007 were used in this study. The maximum parsimony tree comprising 228 amino acid changes revealed that there are 8 subgroups from 2000 to 2007 in South Korea (Fig. 2A). Three isolates of 1999 and two of the 2002 season were nested between A/Beijing/32/1992 and A/Sydney/5/1997. The other three isolates of 1999 formed a separate clade from A/Moscow/10/1999. In 2002, 12 of 16 isolates were Fujian/411-like, excluding A/SouthKorea/C5-4/2002 and A/Kwangju/219/2002 (Fig. 2A). Five isolates of the 02-03 group were found to possess amino acid sequences essentially identical with those of A/Fujian/411/2002, and an additional three isolates could be regarded as identical if the missing or ambiguous sequences were supposed to be the same as the Fujian sequence. This suggests that the Fujian strains were introduced and widespread prior to the 2002-2003 season in South Korea.

A reassortant strain emerged in New Zealand and Australia in the summer of 2003 and was later spread to the United States and Europe during the 2003-2004 season (2, 4, 13). The epidemiological and genetic data of the Fujian-like influenza viruses, including 30 New York strains (19), indicated that both the reassortants and nonreassortants circulated in Asia during the 2002-2004 seasons (Fig. 3A and C). All of the 11 reassortant New York strains were separated from the nonreassortant New York strains and formed a clade (Fig. 3, gray-lined branches in a circle) with some Asian strains in both the HA and NA trees (Fig. 3B and D). The reassortment event was confirmed by the incongruence of the two trees, i.e., the deep branching between 11 reassortants and A/NewYork/406/2002 (marked as a gray dot on the trees in Fig. 3) which had ap-

FIG. 1. Weekly influenza epidemic time series. (A) Influenza virus isolates in South Korea over the period from 2000 to 2007. Different subtypes are shown in different gray shades. (B) Epidemics in South Korea and United States. The y axis shows influenza-like illness over the period from 2000 to 2007. (C) Proportions of influenza viruses A/H1N1, A/H3N2, and B in South Korea (KOR) and the United States in each season. Unsubtyped influenza A viruses of the United States were assumed to have proportions identical to those of subtyped ones.

peared prior to the reassortants in the HA tree and the close relationship among them in the NA tree. The reliability of the reassortant clade was supported not only by the moderate aLRT values (0.785 and 0.983 for the HA and NA trees, respectively) but also by the fact that no incongruence was found between both trees (i.e., no strain was located in the reassortant clade in the HA tree and simultaneously positioned in the nonreassortant group in the NA tree, or vice versa).

Unlike the Fujian reassortants, all of the South Korean strains were separated from the Fujian-reassortant clade with the exception of two strains, A/Korea/124/2003 and A/Jeju/218/2004 (Fig. 2A). The phylogenetic tree of HA demonstrated that the nonreassortant strains rapidly predominated in South Korea as well as in Asia during the 2002-2004 seasons (Fig. 3A). While the reassortment event was known to be the source of increased fitness of the virus in Australia, the United States, and Europe, it is evident that the nonreassortants became the predominant strains in the following seasons. The Fujian reassortants were indeed significantly reduced in the 2004-2005 season and disappeared (data not shown). In this context, it is not surprising to observe that the epidemic strains to date were descended from the nonreassortant lineage. Our results thus strongly suggest that the antigenic drift of HA alone gave sufficient fitness increase to the virus in and outside Asia (Fig. 3A and 4A). The 06-07A and 06-07B clades of the nonreassortants were predominant during the 2006-2007 season, and the recent strains originated from the minor clade which had the G50E mutation in the 2005-2006 season.

Amino acid variation. A total of 105 sites were found to be subjected to amino acid substitutions, which were marked retrospectively on the maximum parsimony tree from A/Sydney/5/1997 to 2007 strains, excluding uninformative amino acid changes (Fig. 2). It is clearly evident that a rapid evolution which had occurred between 2001 and 2002 resulted in the emergence of the Fujian/411 strain. These include antigenic sites A(Ile144), B(His155 and Ser186), and E(Glu83), a receptor binding site (Gly225), and the positive selection sites (Gln156 and Ser186), which also contribute to receptor-binding adaptation (5, 18, 27). Multiple changes were also found in amino acid residues 50, 142, 144, and 225. The R50G mutation took place in the 00-01 group, with additional changes in the clade containing A/Brisbane/10/2007 and 2009 strains. The amino acid residue 144, located at antigenic site A, was altered from Ile to Asn between 1999 and 2001. Additional mutations simultaneously occurred in some of the 03-04 and 06-07A strains from Asn to Asp. Amino acid residue 225 was mutated twice at the emergences of the 02-03 and 05-06 strains. In the 2006-2007 season, Arg142 was mutated far back to its original glycine residue, which could be found in A/Sydney/5/1997.

Origin of A/Fujian/411/2002. We reconstructed a phylogenetic tree to estimate the position of A/Fujian/411/2002 among 521 strains isolated from around the globe in 2001 and 2002 (Fig. 4A) and chose 53 strains located near the emergence of A/Fujian/411/2002. Sequential amino acid changes at key antigenic sites along the evolutionary pathway of the Fujian strains are shown in the maximum likelihood tree (Fig. 4B). The S186G and A131T mutations occurred in 2001 and were followed by mutations of both L25I and H75Q. Since any intermediate strain having one mutation of either L25I or

H75Q was not observed, the order of the two mutations could not be determined. The phylogenetic position of A/India/C3-45/2002 and A/Taiwan/8/2002, which have mutations L25I and H75Q but not H155T, suggested that the H155T mutation occurred after the population spread of the simultaneous mutations of L25I and H75Q. The phylogenetic tree also indicated that A/Fujian/411/2002 already has additional DNA substitutions from the trunk strains (A/Hunan/407/2002, A/Cheju/311/2002, A/Chungnam/447/2002, and A/Kyongnam/347/2002), despite their identical amino acid sequences.

Close comparative analyses of the sequences and amino acid changes revealed a couple of prominent features. First, most of the intermediates and Fujian-like strains were isolated from Asian countries, whereas 7 out of 53 strains were not Asian. In addition, none of the earliest strains of each clade were isolated from non-Asian countries. Given the sampling bias that only 33.7% (175 of 521) are isolated in Asia (Fig. 4A), our results strongly suggest that these mutational events associated with the Fujian strains took place in Asia. Second, the sequences of the isolates collected during the 2001-2002 season allowed us to estimate the evolutionary history and inferred date of introduction to the Asian population of the Fujian strains. Closely dated phylogeny from 26 December 2001 through 11 August 2002 showed that the antigenic evolution of the H3N2 Fujian strains had periods of rapid antigenic changes, equivalent to 10 amino acid changes per year (Fig. 4C). Different subtypes evolve at different rates, such that H3N2 viruses change more rapidly than H1N1 viruses, with an average rate of 3.6 amino acid substitutions per year (24). In this regard, the change in the 14 amino acids that had accumulated from the Moscow/10/1999 clade to the Fujian/411/2002 clade showed an exceptionally high rate of evolution. However, the genetic distance of vaccine strains from a clade to a subsequent clade was merely two (A/Wellington/01/2004 to A/California/7/2004, or A/California/7/2004 to A/Wisconsin/67/2005) or three amino acid substitutions (A/Sydney/5/1997 to A/Moscow/10/1999, or A/Fujian/411/2002 to A/Wellington/01/2004). Taken together, our results demonstrated that the antigenic evolution of the Fujian strains was initiated by rapid antigenic change that occurred in Asia, which then continued as relatively modest changes.

DISCUSSION

Human influenza H3N2 viruses have been the dominant strain in most years since they first emerged in 1968 and have been responsible for one of the most serious respiratory infections until the novel swine-origin influenza H1N1 virus emerged in 2009, causing a new pandemic (28). The phylogenetic tree of 269 HA sequences of human influenza H3N2 viruses collected in South Korea showed that the viral genes

FIG. 2. Phylogenetic analysis and amino acid substitutions of HA of influenza A/H3N2 viruses circulating in South Korea from 1999 to 2007. (A) The maximum parsimony tree. Reference vaccine strains are highlighted in gray. Six 2009 strains from the United States and the Philippines are combined. Amino acid substitutions in major clades are described under the internal branches, and the substitutions to extant lineage are highlighted in bold. Only one representative strain is presented (in bold) along with the number of identical strains when there were more than two strains having identical amino acid sequences. Numbers in parentheses under group names indicate the number of isolates in that group. (B) Locations of the amino acid substitutions that were found in major clades on an HA structure model (Protein Data Bank [PDB] identification no. 1HA0).

FIG. 3. Maximum likelihood (ML) phylogenies reconstructed by PhyML program to identify the Fujian reassortants in Asia from 2002 to 2004. The clade A strains shown in Fig. 1 of reference 19 were used for the reference sequences of reassortants. The closely related nonreassorted strain of clade A, A/NewYork/406/2002, is marked as a gray dot on the ML trees. The node support values for reassortant clades were estimated using an approximate likelihood ratio test incorporated in PhyML (1). (A) ML tree of HA1 nucleotide sequences of 637 Asian strains, 30 New York strains, and A/Moscow/10/1999 as a phylogenetic root. The Fujian/411 clade is shown, and the branch marked by a circle indicates a reassortant clade. (B) The reassortant clade shown in panel A. (C) ML tree of neuraminidase (NA) nucleotide sequences of 161 Asian strains, 30 New York strains, and two outgroup strains (A/NewYork/313/1998 and A/NewYork/328/1998). (D) The reassortant clade shown in panel C.

FIG. 4. Maximum likelihood (ML) trees and timeline of the emergence of the A/Fujian/411/2002 strain. Both trees are rooted by A/Moscow/10/1999. (A) The ML tree of HA1 domain nucleotide sequences of 521 human influenza A/H3N2 virus strains isolated worldwide from 2001 to 2002. The circle indicates the locations of the strains for the further analysis shown in panel B. (B) The ML tree of 53 strains around the emergence of the Fujian/411 strain. Two vaccine strains isolated in 2004 and A/Moscow/10/1999 as a root were included. The earliest isolates of the clades are highlighted in bold. The amino acid changes are described under the internal branches. (C) Timeline of the appearance of A/Fujian/411/2002 in Asia. The earliest day of each clade is described under the timeline.

formed seasonal phylogenetic clusters which have evolved from A/Beijing/32/1992 strains to A/Brisbane/10/2007 strains via A/California/7/2004 strains from 1999 to 2007. The results indicated that progressive antigenic drift occurred at the HA antigen in these seasons. Notably, the strains of different clusters often cocirculated within the same season, which was most apparent with the identification of multiple subclades of H3N2

viruses in 1999 and 2002. Moreover, as shown in Fig. 1A, seasonal H1N1, H3N2, and B viruses have circulated simultaneously during the seasons.

A change of dominant strains in H3N2 from A/Moscow/10/1999 to A/Fujian/411/2002 caused a worldwide epidemic, since the H3N2 vaccine strain for the 2002-2003 season (A/Moscow/10/1999) did not antigenically match the circulating A/Fujian/

411/2002-like viruses, reducing effectiveness against virus-caused illness (3, 14, 22). The emergence of A/Fujian/411/2002-like strains thus coincided with a higher level of influenza-related morbidity, and the 2002-2003 season was clearly a turning point, with regard to circulating influenza H3N2 viruses in Asia. The Fujian-like strains underwent a rapid change in amino acid sequence of HA in the 2001-2002 season, and relatively slow and constant antigenic changes were subsequently observed from 2003 to 2007. Interestingly, a reassortant strain emerged early in the New Zealand winter, followed by the appearance of similar viruses in Australia (2), which was later seen in the United States, Europe, and Brazil during the 2003-2004 season. While the HA sequence of these viruses demonstrated only minor differences from the Fujian H3N2 strain, NA and other internal genes (NS, NP, and M) were different from those of circulating nonreassortant H3N2 viruses (Fig. 3A and C) (13). Both the reassortant and nonreassortant viruses circulated not only in the United States and Europe but also in Asia, possibly due to the reintroduction of the reassortant strains from the Southern hemisphere. Nevertheless, the nonreassortant strains, not the reassortants, became predominant in the following years.

It was not known what features of the A/Fujian/411/2002 strains were responsible for the global spread and how the nonreassortant strains were again dominant against the reassortant ones. The 2001-2002 season strains matched the A/Moscow/10/1999 vaccine strain. Remarkably, a total of 14 amino acid changes (plus 4 amino acid changes from the most common recent ancestor of A/Moscow/10/1999 and A/Fujian/411/2002 to the Moscow strain) were found across the antigenic sites of the HA1 domain, which distinguished the Fujian strain from the Moscow strain (Fig. 2A and B). It was previously reported that the locations of a total of 26 amino acid changes in the *in vitro* mutants matched those at which mainstream amino acid changes had occurred in HA from 1968 to 2000 (20). In contrast, most mutations in the Fujian strain appeared in a short period of time from December 2001 to August 2002 (Fig. 3C). Among the 14 amino acid changes, 10 of them were located at one of the five antigenic sites: H75Q and E83K (site E); A131T and I144N (site A); H155T, Q156H, S186G, and T192I (site B); and D172E and W222R (site D). It was shown that two residues, 155 and 156, are responsible for the major antigenic differences between the A/Moscow/10/1999 and A/Fujian/411/2002-like strains (13). H155T and Q156H were indeed present in the isolates from the 2002-2003 season, whereas some intermediate isolates with the replacement at site 155 but not at site 156 were also identified from the same season.

In the 2003-2004 season, the South Korean nonreassortant strains had at least three additional amino acid substitutions in antigenic sites B and D, namely, Y159F, S189N, and S227P, from the Fujian/411 strain, whereas two reassortants had only one or two mutations from the Fujian strain. The reassortants were found to have eventually faded away, suggesting that the nonreassortant viruses were more antigenically advanced than the reassortants. The epidemic of the Fujian-like strains during the previous season might have hampered the introduction of reassortant strains to Asia since the reassortant strains gained scant antigenic differences from the original Fujian/411 HA. It was recently reported that newly dominant A/California/7/

2004-like strains, which featured two key amino acid changes in the polymerase PA segment, grew to higher titers in MDCK cells (19). Influenza virus strains thus can be selected through mutations in replicative fitness and virulence. Taken together, these findings strongly suggest that the collapse of the reassortants is caused mainly by the preceding antigenic change of nonreassortant A/California/7/2004-like strains.

The isolates collected during successive seasons were further observed to undergo a progressive antigenic drift from A/California/7/2004-like strains to A/Brisbane/10/2007-like strains. Both A/Fujian/411/2002-like and A/California/7/2004-like viruses were prevalent in the 2004-2005 season, as A/California/7/2004-like viruses circulated as a new strain. There were 8 amino acid differences between A/Fujian/411/2002 and A/California/7/2004. The 5 mutations which were commonly found in 2004-2005 isolates (A/California/7/2004-like) were located in antigenic sites: K145N (site A), Y159F and S189N (site B), and V226I and S227P (site D). A/California/7/2004-like viruses then became the new predominant strains in the successive seasons. In the 2005-2006 season, S193F and D225N substitutions were accumulated in the H3N2 strains, and the R142G mutants dominated during the 2006-2007 season. However, it was notable that recent strains were descended from a minority group of the 2005-2006 season which has a G50E mutation.

A/Fujian/411/2002 was first collected in August 2002 in China, and a Fujian strain in South Korea was first detected in Busan on 20 November 2002, and simultaneous appearances throughout South Korea followed. Notably, the 2002 FIFA World Cup was held in South Korea and Japan from 31 May to 30 June 2002. And the 14th Asian Games were held in Busan, South Korea, from 29 September to 14 October 2002, with a total number of 18,000 athletes and officials from 44 countries. Asian countries have intensive contact through air travel, which could contribute to viral transmission patterns. Recent analysis of air-traffic patterns showed a strong correlation between the international travel and 2009 H1N1 transmission (16). It was proposed that the variability of influenza H3N2 epidemics may form an east-southeast Asian circulation network that maintains influenza virus in the region by passing from epidemic to epidemic (22). A network of monitoring efforts for international events can be employed in preparation of a novel influenza outbreak.

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