

RESEARCH ARTICLE

Molecular Epidemiology and Phylogenetic Analysis of Human Adenovirus Caused an Outbreak in Taiwan during 2011

Yung-Cheng Lin^{1,2}, Po-Liang Lu³, Kuei-Hsiang Lin⁴, Pei-Yu Chu⁵, Chu-Feng Wang⁶, Jih-Hui Lin^{7*}, Hsin-Fu Liu^{1,2,8*}

1 Department of Bioscience and Biotechnology, National Taiwan Ocean University, Keelung, Taiwan, **2** Department of Medical Research, Mackay Memorial Hospital, Taipei, Taiwan, **3** Division of Infectious Diseases, Department of Internal Medicine, Kaohsiung Medical University Hospital, Kaohsiung, Taiwan, **4** Department of Clinical Laboratory, Kaohsiung Medical University, Kaohsiung, Taiwan, **5** Department of Medical Laboratory Science and Biotechnology, Kaohsiung Medical University, Kaohsiung, Taiwan, **6** Department of Laboratory Medicine, Kaohsiung Medical University Hospital, Kaohsiung Medical University, Kaohsiung, Taiwan, **7** Center for Research, Diagnostics and Vaccine Development, Centers for Disease Control, Taipei, Taiwan, **8** Center for General Education, National Taipei University of Nursing and Health Sciences, Taipei, Taiwan

* hsinfu@mmh.org.tw (HFL); jeffy320@cdc.gov.tw (JHL)



OPEN ACCESS

Citation: Lin Y-C, Lu P-L, Lin K-H, Chu P-Y, Wang C-F, Lin J-H, et al. (2015) Molecular Epidemiology and Phylogenetic Analysis of Human Adenovirus Caused an Outbreak in Taiwan during 2011. PLoS ONE 10 (5): e0127377. doi:10.1371/journal.pone.0127377

Academic Editor: Massimo Ciccozzi, National Institute of Health, ITALY

Received: January 30, 2015

Accepted: April 15, 2015

Published: May 18, 2015

Copyright: © 2015 Lin et al. This is an open access article distributed under the terms of the [Creative Commons Attribution License](http://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Data Availability Statement: All relevant data are within the paper and its Supporting Information files.

Funding: This work was supported by the Ministry of Science and Technology, Taiwan (grant 102-2314-B-195-013-MY2) (<http://www.most.gov.tw/mp.aspx?mp=7>), Mackay Memorial Hospital, Taiwan (grant MMH-101113 and MMH-10226) (<http://eng.mmh.org.tw/dhnl>). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing Interests: The authors have declared that no competing interests exist.

Abstract

An outbreak of adenovirus has been surveyed in Taiwan in 2011. To better understand the evolution and epidemiology of adenovirus in Taiwan, full-length sequence of hexon and fiber coapsid protein was analyzed using series of phylogenetic and dynamic evolution tools. Six different serotypes were identified in this outbreak and the species B was predominant (HAdV-3, 71.50%; HAdV-7, 15.46%). The most frequent diagnosis was acute tonsillitis (54.59%) and bronchitis (47.83%). Phylogenetic analysis revealed that hexon protein gene sequences were highly conserved for HAdV-3 and HAdV-7 circulation in Taiwan. However, comparison of restriction fragment length polymorphism (RFLP) analysis and phylogenetic trees of fiber gene in HAdV-7 clearly indicated that the predominant genotype in Taiwan has shifted from 7b to 7d. Several positive selection sites were observed in hexon protein. The estimated nucleotide substitution rates of hexon protein of HAdV-3 and HAdV-7 were 0.234×10^{-3} substitutions/site/year (95% HPD: $0.387 \sim 0.095 \times 10^{-3}$) and 1.107×10^{-3} (95% HPD: $0.541 \sim 1.604$) respectively; those of the fiber protein of HAdV-3 and HAdV-7 were 1.085×10^{-3} (95% HPD: $1.767 \sim 0.486$) and 0.132×10^{-3} (95% HPD: $0.283 \sim 0.014$) respectively. Phylodynamic analysis by Bayesian skyline plot (BSP) suggested that using individual gene to evaluate the effective population size might possibly cause miscalculation. In summary, the virus evolution is ongoing, and continuous surveillance of this virus evolution will contribute to the control of the epidemic.

Introduction

Human adenoviruses (HAdVs) are double-stranded non-enveloped DNA viruses belonging to the family *Adenoviridae*, genus *Mastadenovirus*. More than sixty serotypes of HAdVs have been recognized and classified into seven species (A-G) based on genome sequencing, phylogenetic and biological characteristics [1, 2]. Capsid proteins such as hexon and fiber in HAdV play a critical role of entry into cell and immune response as well [3]. Antigenic and genetic variability of these regions can cause epidemics or outbreaks [4–6]. HAdV are implicated in a wide range of human diseases, including respiratory diseases, conjunctivitis, cystitis and gastroenteritis. Acute respiratory tract infection (ARTI) is a serious threat to infant and child who usually required hospitalization. Species B (HAdV-3, 7, 14, 55), C (HAdV-1, 2, 5, 6) and E (HAdV-4) are frequently isolated from pediatric patients with ARTI [4, 6–8]. In particular the HAdV-7 is frequently associated with the severe ARTI such as lethal pneumonia or bronchopneumonia [9, 10]

HAdV is one of the major pathogens of the ARTI in Taiwan [11]. A total of 3 outbreaks caused by this virus had been detected in Taiwan since 1999. HAdV-7 was responsible for the outbreak in 1999, HAdV-4 in 2000~2001, and HAdV-3 in 2004~5 [12, 13]. After the outbreak in 1999, isolation rate of HAdV-7 became lower. However, HAdV-3 was still the most common serotype during the past two decades [14]. In 2011, clinical isolates of adenovirus was significantly increased implying an outbreak of HAdV. To investigate the predominant serotype of the virus in this outbreak, and to reveal whether the predominant strains have antigenic or sequence variation in the hexon and/or fiber capsid protein gene, we conducted a comprehensive phylogenetic and evolutionary analysis. The clinical features of the adenovirus infections were also analyzed.

Materials and Methods

Ethics Statement and Study Design

A total of 207 isolates used in this study were stratified random sampling depending on number of monthly adenovirus positive case from positive stocks collected during the outbreak in 2011. Virus was isolated from either nasopharyngeal aspirate or throat swabs from children with ARTIs in Kaohsiung Medical University Chung-Ho Memorial Hospital, and then grown in H292 and A549 cells (purchased from American Type Culture Collection). The study of ethical approval was obtained from Kaohsiung Medical University Hospital Institutional Review Board (KMUH-IRB-980344). This was a retrospective study without intervention or obtaining extra clinical specimens. All samples were de-identified and analyzed anonymously, so informed consent was waived. The Institutional Review Board of Kaohsiung Medical University Hospital also approved the waiving of informed consent. Statistical analyses of the correlation between serotypes and clinical data were using JMP software (Version 8). The statistical significance was set at the level of $p < 0.05$.

Serotype and genotype classification

Viral DNA was extracted using QIAamp DNA Mini Kit (Qiagen, Santa Clara, CA). For restriction fragment length polymorphism (RFLP) analysis, DNA extraction was according to the traditional phenol/chloroform/isoamyl alcohol (25:24:1) extraction methods [15]. DNA was stored at -80°C until use. The primer pair AdnU-S' (5'-TTCCCCATGGCNCACAACAC-3') and AdnU-A (5'-GCCTCGATGACGCCGCGGTG-3') were used to amplify a 956-bp product from the hexon region [16]. PCR products were subjected to sequence assay and were used for the identification of serotypes. For genotyping, aliquots containing 1–2 μg of viral DNA were

digested with 10–15 U of BamHI, Bcl I, BstE II and Bgl II (Promega, Madison, WI, USA) according to the manufacturer's instructions. Digested products were electrophoresis on 0.8% agarose gel containing SYBR Green I (Invitrogen, Ltd.) and run for 16 hr at 50 V in TBE buffer. The RFLP patterns were identified according to nomenclature system developed by Li et al. and other modification [17, 18].

PCR amplification and sequencing of hexon and fiber genes

We designed six primers to amplify the full length of hexon and fiber genes. Primers Ad-3F-F (5'-ACCTCACCTCTTCCCAACT-3'), Ad-3F-R (5'-GAAGGGGGAGGCAAATAAC-3'), Ad-7F-F (5'-GAAATTTTCTCCAGCAGCA-3'), Ad-7F-R (5'-GAAGGGGGAGGCAAATAAC-3') were used to amplify the full length HAdV-3 and HAdV-7 fiber gene, respectively. The primer pair of B1-H-F (5'-GCAGCAGAGGAGAAAGGAAG-3') and B1-H-R (5'-GACGATGGCTTTGAGCTCTT-3') was used to amplify the whole hexon gene in both HAdV-3 and HAdV-7. The primer sequence and annealing temperature was shown in supplementary table (S1 Table).

PCR amplification was done by Sensoquest Labcycler (SensoQuest GmbH) with *Pfu* DNA polymerase (Promega Corporation, WI). PCR products were purified with QIAquick spin (Qiagen, Valencia, CA) columns and subject to direct sequencing by BigDye 3.1 Terminator Cycle Sequencing reagents on ABI Prism 3730 DNA Analyzer (Applied Biosystems, Forest City, CA).

Phylogenetic and phylodynamic analysis

Full length of hexon and fiber genes were alignment by Muscle implemented in the MEGA 6 software [19, 20]. Likelihood mapping analysis was performed to evaluate the phylogenetic signal with TREE-PUZZLE software version 5.2 [21]. The transition/transversion ratio, base frequencies, and α parameter of gamma distribution were estimated by TREE-PUZZLE software version 5.2. Phylogenetic trees were reconstructed with the neighbor-joining (NJ) and maximum likelihood (ML) methods using the MEGA 6 and PhyML 3.0 [19, 22]. The robustness of the phylogenetic trees was statistically evaluated by bootstrap analysis with 1000 replicates. The bootstrap value >75% was considered to a monophyletic group.

The evolution rates and population size changes of HAdV were determined using Bayesian Markov Chain Monte Carlo (MCMC) method offered in BEAST v1.8.2 along with the BEAGLE library [23, 24]. The SRD06 nucleotide substitution model was used in all simulations as this model is recognized to provide better resolution for coding regions to Bayesian analysis [25]. The demographic model, included Bayesian skyline, constant size, exponential growth, logistic growth, and expansion growth was used to estimate evolutionary and population dynamic, under both molecular clock models (strict and relaxed) [23]. The best fit of demographic and clock model was estimated from model comparison by Akaike's information criterion (AICM) in the Tracer program v1.6 [26]. The MCMC chains were run for sufficient time to achieve convergence (ESS>200). In addition, the uncertainty of parameter is estimated in 95% highest probability density (HPD). The Maximum Clade Credibility (MCC) tree was constructed by Tree Annotator v 1.7.4, with the 10% burn-in. The final of phylogenetic trees were edited by Figtree v1.4.2.

Selection pressure of hexon and fiber protein genes

To determine the selection pressures on hexon and fiber protein of HAdV, we estimated the ratio of non-synonymous substitutions (dN) and synonymous substitutions (dS) per site based on ML trees under the appropriate substitution model, using the single likelihood ancestor

Table 1. Demographic and clinical data for adenovirus-positive patients according to each serotype.

| Variables | Total HAdV-positives | Number of adenovirus serotypes (%) | | | | | |
|---------------------------|----------------------|------------------------------------|------------|------------|------------|------------|--------------|
| | | HAdV—1 | HAdV—2 | HAdV—3 | HAdV—5 | HAdV—6 | HAdV—7 |
| Serotype | 207 | 6(2.89) | 16(7.73) | 148(71.50) | 2(0.97) | 3(1.45) | 32(15.46) |
| Sex | | | | | | | |
| Male | 82(60.0) | 3(50.0) | 5(31.25) | 93(62.84) | 1(50.0) | 2(66.67) | 23(65.63) |
| Female | 125(40.0) | 3(50.0) | 11(68.75) | 55(37.16) | 1(50.0) | 1(33.33) | 11(34.38) |
| Age | 5.12±4.08 | 2.33±1.37 | 2.53±1.31 | 5.21±3.76 | 3.75±3.18 | 3.0±0.0 | 6.77±5.79 |
| Clinical presentation | | | | | | | |
| Length of hospitalization | 6.21±3.15 | 5.33±3.39 | 5.56±1.93 | 6.40±3.30 | 5.0±0.0 | 6.0±1.00 | 5.91±3.14 |
| Fever (°C) | 38.88±2.87 | 38.57±0.96 | 38.96±0.93 | 39.13±0.94 | 39.05±0.49 | 39.17±0.29 | 37.72±0.6.92 |
| Fever (days) | 3.62±2.35 | 5.50±5.01 | 4.19±2.37 | 3.38±1.95 | 2.50±0.71 | 1.67±0.58 | 4.35±3.11 |
| Fever | 199(96.14) | 6(100.00) | 16(100.00) | 142(95.95) | 2(100.00) | 3(100.00) | 30(93.75) |
| Cough | 158(76.33) | 5(83.33) | 13(81.25) | 112(75.68) | 2(66.67) | 24(75.00) | 158(76.33) |
| Rhinorrhoea | 126(60.87) | 4(66.67) | 9(56.25) | 91(61.49) | 2(100.00) | 2(66.67) | 18(56.25) |
| Diarrhea | 71(34.30) | 0(0.00) | 4(25.00) | 52(35.14) | 1(50.00) | 1(33.33) | 13(40.63) |
| Clinical presentation | | | | | | | |
| Acute tonsillitis | 113(54.59) | 4(66.67) | 11(68.75) | 81(54.73) | 1(50.00) | 3(100.00) | 13(40.63) |
| Acute pharyngitis | 22(10.63) | 1(16.67) | 1(6.25) | 15(10.14) | 0 | 0 | 5(15.63) |
| Acute sinusitis | 36(17.39) | 1(16.67) | 3(18.75) | 29(19.59) | 0 | 0 | 3(9.38) |
| Bronchopneumonia | 53(25.60) | 2(33.33) | 4(25.00) | 37(25.00) | 0 | 2(66.67) | 8(25.00) |
| Bronchitis | 99(47.83) | 4(66.67) | 5(31.25) | 73(49.32) | 2(66.67) | 2(66.67) | 13(40.63) |
| Pneumonia | 26(12.56) | 0 | 2(12.50) | 21(14.19) | 0 | 0 | 3(9.38) |
| Gastroenteritis | 28(13.53) | 0 | 2(12.50) | 23(15.54) | 0 | 0 | 3(9.38) |

doi:10.1371/journal.pone.0127377.t001

counting (SLAC), fixed effects likelihood (FEL) methods with significance level on 0.05. Bayesian tests for selection acting on individual sites were using FUBAR with posterior probabilities on 0.95 [27]. The directional evolution in protein sequences (DEPS) test was using to detect selective sweeps [28]. Residues with a Bayes factor of >100 were reported as positively selected.

All methods were implemented in the HyPhy package and accessed through the Datamonkey web-server interface (<http://www.datamonkey.org>) [29, 30].

Results

Serotypes and clinical features

A total of 207 isolates were analysis by amplify partial hexon sequences and followed by BLAST search on NCBI database (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>). All isolates were included in the clinical feature analysis. Among the 2011 outbreaks, six different serotypes were identified and the species B were the predominant strains (HAdV-3, 71.50%; HAdV-7, 15.46%) (Table 1). We only focus on these two major serotypes in this study.

The most common symptoms were fever (96.14%), cough (76.33%) and rhinitis (60.87%). The most frequent diagnosis was acute tonsillitis (54.59%) and bronchitis (47.83%). The male-to-female ratio is 1.5:1; the mean age of the patients was 5.12 ± 4.08 years. The mean peak body temperature and length of hospitalization was 38.88 ± 2.87°C and 6.21±3.15 days respectively. The age distribution and fever days showed significant difference in patterns depending on the HAdV serotypes ($P = 0.007$ and 0.032). Furthermore, HAdV-3 presented higher fever than that of HAdV-7 ($P = 0.011$) (Table 1).

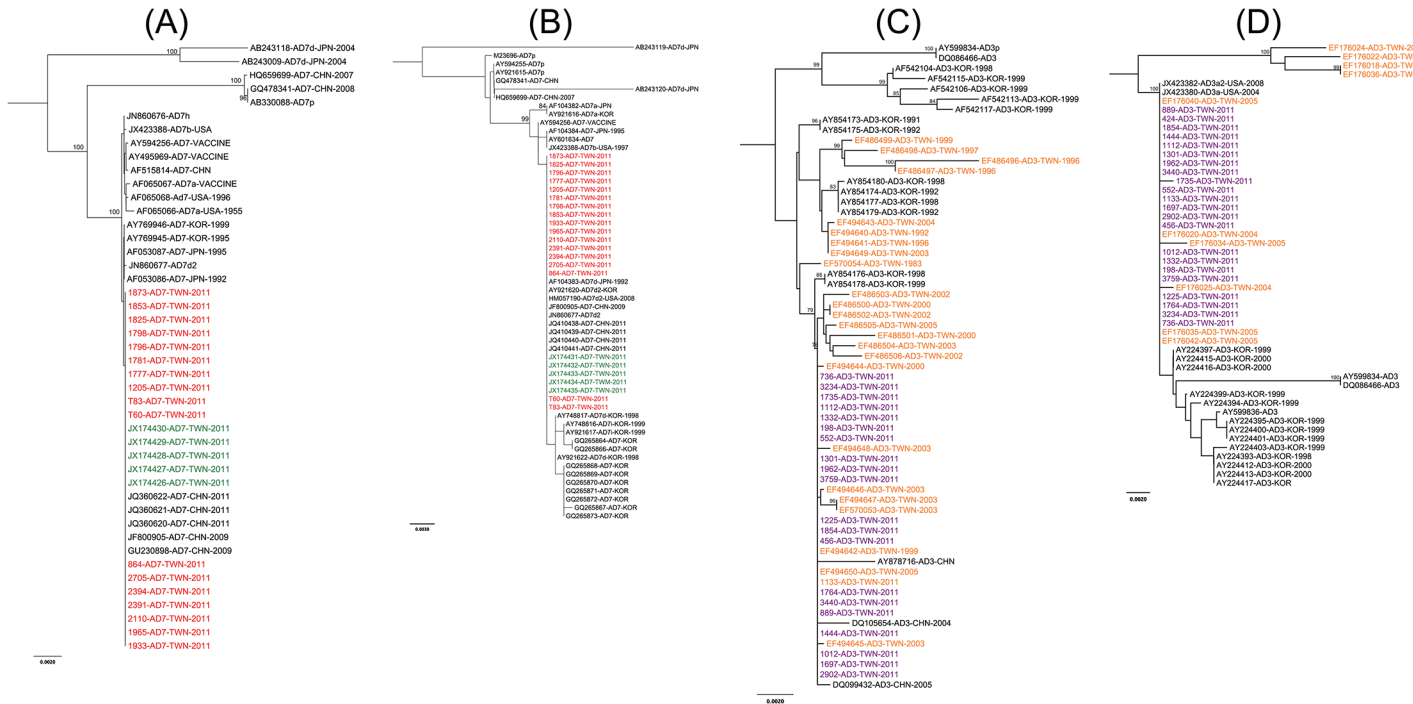


Fig 1. Phylogenetic analysis of the human adenovirus type 3 and type 7 hexon and fiber genes. The phylogenetic tree was inferred from HAdV-7 hexon gene (A), HAdV-7 fiber gene (B), HAdV-3 hexon gene (C) and HAdV-3 fiber gene (D). Tree topology was constructed using the neighbor-joining method. The topologic accuracy of the tree was evaluated by using 1,000 bootstrap replicates. Only bootstrap values greater than 75% are shown. Red and purple texts represent HAdV- 7 and 3 isolated in Taiwan in this outbreak, respectively. Orange text represents HAdV- 3 reference sequences from Taiwan. Green text was HAdV- 7 reference sequences from Northern Taiwan.

doi:10.1371/journal.pone.0127377.g001

Phylogenetic analysis of the hexon and fiber genes

Likelihood mapping analysis showed that most of the quartets were located in the three corners of the triangle suggesting a treelike signal. Consistent tree topologies were observed by NJ and ML methods. The analyzed HAdV-7 hexon gene sequences were highly conserved and phylogenetic analysis showed they were clustered together with reference sequences from NCBI database and the strains isolated in same period from north Taiwan [31], except prototype and two 7d isolates from Japan in 2004 (with 96.3% and 95.4% identity respectively) (Fig 1A). For the fiber gene, our results showed that HAdV-7 in this outbreak were all classified into a single cluster with HAdV-7d2 and 7d rather than the previous predominant genotype 7b (Fig 1B). To confirm this observation, whole genome viral DNA was analyzed by RFLP and compared with the previous report [13, 17, 18]. These restriction patterns of Bam HI showed the HAdV-7 in this outbreak was belong to 7d or 7d2 genotype. Furthermore, three restriction enzymes (BstE II, Bcl I and Bgl II) conformed that they were not 7d2 genotype (S1 Fig). Based on these results we suggest the predominant of HAdV-7 in Taiwan has shifted from 7b to 7d genotype.

Similar results were observed from HAdV-3, all hexon and fiber gene sequences were highly conserved and also clustered together with our previously reported strains and other reference strains from Taiwan (Fig 1C and 1D). The viral nucleotide sequences determined in this study have been assigned with GenBank accession numbers KC456083 to KC456103 (HAdV-3 hexon genes), KC456104 to KC456125 (HAdV-3 fiber genes), KC456126 to KC456142 KC456125 (HAdV-7 fiber genes) and KC456143 to KC456159 (HAdV-7 hexon genes). The accession numbers of sequences used for phylogenetic and evolutionary analysis in this study were listed in supplementary table (S2 Table).

Table 2. Selection sites detected in hexon and fiber of HAdV- 3 and 7.

| | Positively selected sites | | | | No. of negatively selected sites | | | Mean d _N /d _S |
|----------------|---------------------------|------------------|-------------------|--|----------------------------------|-----|------|-------------------------------------|
| | SLAC ^a | FEL ^a | FUBA ^b | DEPS ^c | SLAC | FEL | FUBA | |
| HAdV- 3 | | | | | | | | |
| Hexon | Non | 649 | 137,205,649 | 22,205,254,299,326,386,417,429,439,649,651,652,667,675,714 | 2 | 5 | 4 | 0.520 |
| Fiber | Non | Non | Non | Non | Non | 3 | 3 | 0.534 |
| HAdV- 7 | | | | | | | | |
| Hexon | 146 | Non | Non | 443 | 6 | 44 | 27 | 0.092 |
| Fiber | Non | Non | Non | 104 | Non | 3 | 3 | 0.142 |

^a P value of <0.05.

^b Posterior probability of ≥0.95.

^c Bayes factor of >100

doi:10.1371/journal.pone.0127377.t002

Selection pressures in the HAdV- 3 and 7 surface proteins

Selection pressures of hexon and fiber protein in HAdV-3 and 7 were estimated by the dN/dS ratio. The criteria were 1) the ratio of dN/dS < 1 as negative selection, 2) dN/dS = 1 as neutrality, and 3) dN/dS > 1 as positive selection. The mean ratio of dN/dS in HAdV-3 and 7 hexon, fiber protein was 0.520, 0.534 and 0.092, 0.142, respectively (Table 2).

All of open reading frames contained negatively selected codons. The Hexon of HAdV-7 revealed relatively higher negatively selected codons than others. Positive selection was detected on HAdV-3 hexon protein at codon 649 by FEL method. Three codons (137, 205, 649) were detected by FUBA whereas other 15 codons were detected by DEPS.

For HAdV-7, two positive selection sites were detected in hexon protein of which codons 146 by SLAC method and codons 443 by DEPS. A positive selection site was detected in fiber at codons 104 by DEPS method.

Phyldynamic of adenovirus

Phylogenetics of HAdV-3 and HAdV-7 was estimated by Bayesian skyline plot basis on hexon and fiber gene, respectively. The genetic diversity of the HAdV-7 population remained steady until 2005, subsequently, the steep declined the effective population size lasted until 2011 (Fig 2C and 2D). The result of hexon gene in HAdV-3 showed the population was declining after 1999 (Fig 2A). BSP constructed from HAdV-3 hexon, HAdV-7 hexon and fiber were represent downtrend for population dynamics (Fig 2A, 2C, and 2D). However, inconsistent results were found in the HAdV-3 fiber gene. That was steady after 2005 and showing upward trend at after 2010 (Fig 2B).

Evolutionary rates and the most recent common ancestor (tMRCA) of HAdV- hexon and fiber genes

The uncorrelated lognormal relaxed clock model and Bayesian skyline was determined to be a better fit model for both hexon genes. For HAdV-7 fiber gene, the uncorrelated exponential relaxed and Bayesian skyline was the better fit model. For HAdV-3 fiber gene, the better fit model was uncorrelated lognormal relaxed clock model and exponential growth. All of the nucleotide substitution rates, evolutionary rates and tMRCA are summarized in Table 3. The estimated nucleotide substitution rate of hexon protein on HAdV-3 and HAdV-7 was 0.234×10^{-3} substitutions/site/year (95% HPD was $0.387 \sim 0.095 \times 10^{-3}$) and 1.107×10^{-3} (95% HPD was

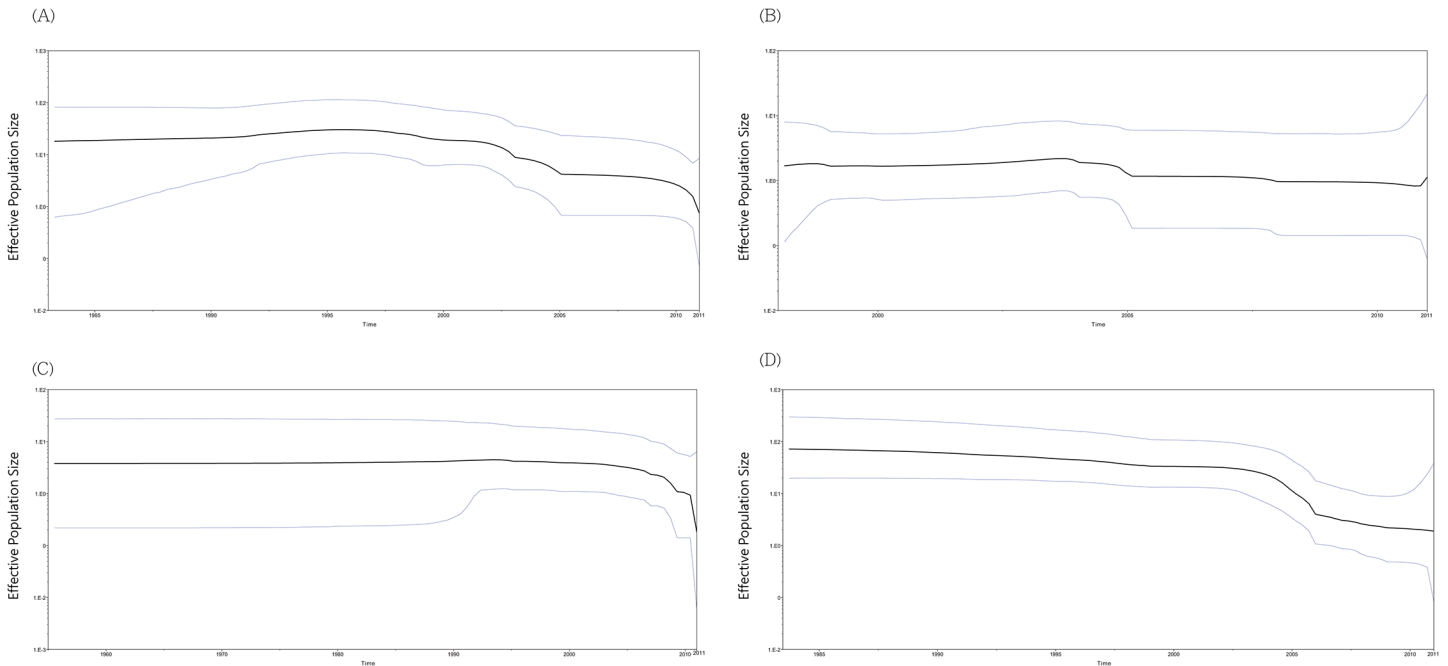


Fig 2. Bayesian skyline plot for complete hexon and fiber gene of the HAdV-3 and HAdV-7. The BSP were inferred from HAdV-3 hexon gene (A), HAdV-3 fiber gene (B), HAdV-7 hexon gene (C) and HAdV-7 fiber gene (D). The bold line represents the median estimate of the effective population size of infections through time, with the 95% HPD values shown within the blue line.

doi:10.1371/journal.pone.0127377.g002

0.541~1.604), respectively. The nucleotide substitution rate of fiber protein on HAdV-3 and HAdV-7 was 1.085×10^{-3} (95% HPD: 1.767~0.486) and 0.132×10^{-3} (95% HPD: 0.283~0.014), respectively. The TMRCA of HAdV-3 was dated to 1964 (95% HPD: 1918~1983) for hexon; the fiber was dated to 1995 (95% HPD: 1991~1998). The TMRCA of HAdV-7 hexon was dated to 1949 (95% HPD: 1931~1955). However, the TMRCA of fiber gene was more than two hundred years (1788, 95% HPD: 1422~1984).

The evolutionary rate of synonymous positions (3rd codon position) was significantly higher than that of nonsynonymous positions (1st and 2nd codon positions) in hexon and fiber genes, except the fiber gene of HAdV-3.

Discussion

Previous studies suggested that viral genetic diversity caused by recombination was considered as the main source of emerging outbreaks [30, 32–34]. For example, HAdV-7h, first isolated from Buenos Aires, Argentina in 1987, had been reported to be highly virulent and predominant only in South America and Japan [8]. Sequence and phylogenetic analysis of HAdV-7h clearly indicated that it was an emerging virus and resulting from the recombination of HAdV-3 fiber [8].

During the last decade, emerging and/or re-emerging adenovirus caused several outbreaks worldwide, including Taiwan, Malaysia, China, the United States, Japan, France, Korea and Portugal [1, 4–6, 12, 13, 32, 35–37]. In 2011, a community outbreak of respiratory tract infections was observed in Taiwan and the majority of the patients required hospitalization. PCR and sequence of partial hexon gene showed that HAdV-3 and HAdV-7 were predominant and constituted this outbreak. The HAdV-3 and HAdV-7 also caused an outbreak in nearby countries China and Korea [38, 39]. The mean age of the patients of this outbreak was 5.12 ± 4.08

Table 3. Mean relative evolutionary rates for codon positions and times of most recent common ancestor (TMRCA) in hexon and fiber gene of Human adenovirus.

| | TMRCA (calendar year) | Substitution rates ($\times 10^{-3}$) subs/site/year | Mean relative substitution rate | SE of mean |
|---------------------------------|--------------------------|---|------------------------------------|---------------|
| HAdV- 3 | | | | |
| Fiber | 1995 (1998~1991) | 1.085 (1.767~0.486) | | |
| 1st codon position (95% HPD) | | | 1.211 (0.7317~1.673) | 1.8918E-3 |
| 2nd codon position (95% HPD) | | | 0.578 (0.2419~0.9669) | 1.5242E-3 |
| 3rd codon position (95% HPD) | | | 1.211 (0.7251~1.669) | 1.8817E-3 |
| Hexon | 1964 (1983~1918) | 0.234 (0.387~0.095) | | |
| 1st codon position (95% HPD) | | | 0.711 (0.4913~0.9411) | 9.1974E-4 |
| 2nd codon position (95% HPD) | | | 0.818 (0.5521~1.1008) | 1.1818E-3 |
| 3rd codon position (95% HPD) | | | 1.471 (1.1008~1.7713) | 1.1912E-3 |
| HAdV- 7 | | | | |
| Fiber | 1788 (1422~1984) | 0.132 (0.283~0.014) | | |
| 1st codon position (95% HPD) | | | 0.422 (0.1741~0.67) | 9.677 E-4 |
| 2nd codon position (95% HPD) | | | 0.504 (0.2447~0.774) | 1.0291 E-3 |
| 3rd codon position (95% HPD) | | | 2.074 (1.7414~2.4109) | 1.2913 E-3 |
| Hexon | 1949 (1931~1955) | 1.107 (0.541~1.604) | | |
| 1st codon position (95% HPD) | | | 0.326 (0.2036~0.4544) | 7.0944 E-4 |
| 2nd codon position (95% HPD) | | | 0.25 (0.1362~0.373) | 6.8106 E-4 |
| 3rd codon position (95% HPD) | | | 2.424 (2.2558~2.5802) | 9.0516 E-4 |

doi:10.1371/journal.pone.0127377.t003

years. The male-to-female ratio (1.5:1) showed that HAdV infections affected more boys than girls. These clinical phenomena are in consistent with previous studies [14, 40, 41]. However, the age distribution showed a significant different pattern depending on the HAdV serotypes, meaning that subgenus B was significantly older than that of subgenus C ($P = 0.007$). This observation was similar to that of HAdV-3 outbreak in Taiwan in 2004~2005 [12].

In Taiwan, HAdV-3a genotype was predominated during the 1983–1999, while HAdV-3a2 was predominated in 2001–2005 [14]. Comparison with phylogenetic trees and sequence alignments of fiber and hexon gene sequences from our previous data indicated that HAdV-3 was highly conserved with other Taiwan strains, and the predominant genotype did not change. According to our surveillance, HAdV-7 was not the major serotype circulating in Taiwan, but

the HAdV-7b genotype was the predominant genotype of HAdV-7 in Taiwan [13]. Interestingly, our phylogenetic analysis showed that all the isolates from this outbreak were clustered with HAdV-7d2 from USA, HAdV-7d from Japan, and HAdV-7d and HAdV-7i from Korea (GenBank accession number HM057190, JN860677, AF104383, AF053087, AY921622 and AY7748816 respectively), rather than previous predominant HAdV-7b. This result suggested that the predominant genotype may have shifted. Further whole genome RFLP analysis supported this hypothesis.

The HAdV-7d, or a closely related variant, HAdV-7d2, was predominant genotype in Japan, Korea and China after 1984 [14, 42, 43], whereas HAdV-7b was predominant genotype in the same period in Taiwan till 2004. After the outbreak in 1999, HAdV-7 isolation rate was decreased in our and other contracted virology laboratories [31]. Interestingly, the transmission route of HAdV-7d that contributed to its emergence remained an important concern. One possible explanation was the frequent travels between Taiwan, China, Japan, Korea, and North American. We hypothesized that HAdV-7d genotype was introduced from an outside source resulting its emergence in 2011. This can be clearly seen in the MCC tree that all isolates from 2011 were clustered with isolates from China in the same period.

Phylodynamics of the hexon gene of HAdV-7 and HAdV-3 showed a downward trend of the effective population size in recent years. This suggested that genetic evolution of hexon gene for both HAdV-3 and 7 were stabilized. However, different results from hexon gene were found in HAdV-3 fiber gene. The effective population size showed on BSP was rise in 2011, which consistent with adenovirus outbreak in 2011.

The amino acid variation associated with positive selection was often observed in hexon gene. Hexon is the surface glycoprotein and thus is an accessible target to antibodies, which could explain the positive selection occurred in there.

For HAdV-3, the amino acid variation (G205V) located within HVR₃ (hypervariable region) was detected as a positive selection site by FUBA and DEPS, while this nonsynonymous substitution was found in a new genotype of HAdV-3 either [44, 45]. The G205V variation might be beneficial to P649 H or R located within HVR₇ also was positive selection site detected by FEL, FUBA, and DEPS. This variation was found in HAdV-3a and HAdV-3a2 subgroups in Taiwan in 1996~1999 and 2002~2005. For HAdV-7, positive selection site was observed in HVR₁ of hexon protein at T146 (436_437 del AC) by SLAC. This amino acid residue only existed in the prototype strain but was deleted in all other HAdV-7 genotypes [46]. This deletion might conducive to HAdV-7 survival. On the other hand, another positive selection at L443Q was found by DEPS. The substitution was from Leu in HAdV-7b to Gln in Ad7d and affected the hydrophobic characteristic (hydrophobic to hydrophilic) [46]. HAdV-7d was replaced HAdV-7b as the predominant circulating virus in our neighboring countries. Therefore, we suggest the L443Q was important codon for HAdV-7d expansion. The relatively low dN/dS ratios in HAdV-7 hexon gene implicated no strong selection occurred in HAdV-7.

This is the first report of dynamic evolution and selection pressure for Adenovirus in Taiwan. In this study, we found that the predominant genotype of HAdV-7 has changed. Although the evolution of the two major capsid proteins was steady, the virus evolution is ongoing under selection pressure. The acquisition of additional mutations in the future could lead to an antigenic drift and cause further outbreaks. In order to quickly respond to an outbreak caused by emergent or re-emergent adenovirus in the future, continuous surveillance of this virus evolution is necessary.

Supporting Information

S1 Fig. Restriction profiles of human adenovirus genome types after digestion with selected enzymes (Bam HI, Bcl I, BstE II and Bgl II) and DNA markers (λ Hind III and ϕ X174 Hinc). M: molecular weight marker; Lane 1–1~1–4 was genotype 7b(2123/99) digestion with Bgl II, BstE II, Bcl I and Bam HI, respectively; Lane 2–1~2–4 was genotype 7d(1205/2011) digestion with Bgl II, BstE II, Bam HI and Bcl I, respectively; Lane 3–1~3–4 was genotype 7d(1777/2011) digestion with BstE II, Bgl II, Bam HI and Bcl I, respectively. (EPS)

S1 Table. Primers used for amplification and sequencing hexon and fiber genes. (DOCX)

S2 Table. The list of references strains used to phylogenetic and evolutionary analysis in this study. (DOCX)

Acknowledgments

We thank all those physicians in sentinel practices that have generously collected the specimens. This study was supported in part by the Ministry of Science and Technology (grant 102-2314-B-195-013-MY2) and Mackay Memorial Hospital, Taiwan (grant MMH-101113 and MMH-10226).

Author Contributions

Conceived and designed the experiments: YCL JHL HFL. Performed the experiments: YCL PLL CFW. Analyzed the data: YCL KHL JHL. Contributed reagents/materials/analysis tools: YCL PLL PYC KHL. Wrote the paper: YCL JHL HFL.

References

1. Robinson CM, Singh G, Henquell C, Walsh MP, Peigue-Lafeuille H, Seto D, et al. Computational analysis and identification of an emergent human adenovirus pathogen implicated in a respiratory fatality. *Virology*. 2011; 409(2):141–7. Epub 2010/11/09. doi: [10.1016/j.virol.2010.10.020](https://doi.org/10.1016/j.virol.2010.10.020) PMID: [21056888](https://pubmed.ncbi.nlm.nih.gov/21056888/); PubMed Central PMCID: PMC3006489.
2. Ferreyra LJ, Giordano MO, Martinez LC, Barril PA, Masachessi G, Isa MB, et al. Tracking novel adenovirus in environmental and human clinical samples: no evidence of endemic human adenovirus type 58 circulation in Cordoba city, Argentina. *Epidemiology and Infection*. 2014;1–5. Epub 2014/08/29. doi: [10.1017/S0950268814002192](https://doi.org/10.1017/S0950268814002192) PMID: [25165987](https://pubmed.ncbi.nlm.nih.gov/25165987/).
3. Russell WC. Adenoviruses: update on structure and function. *The Journal of general virology*. 2009; 90 (Pt 1):1–20. Epub 2008/12/18. doi: [10.1099/vir.0.003087-0](https://doi.org/10.1099/vir.0.003087-0) PMID: [19088268](https://pubmed.ncbi.nlm.nih.gov/19088268/).
4. Walsh MP, Seto J, Jones MS, Chodosh J, Xu W, Seto D. Computational analysis identifies human adenovirus type 55 as a re-emergent acute respiratory disease pathogen. *Journal of clinical microbiology*. 2010; 48(3):991–3. Epub 2010/01/01. doi: [10.1128/JCM.01694-09](https://doi.org/10.1128/JCM.01694-09) PMID: [20042633](https://pubmed.ncbi.nlm.nih.gov/20042633/); PubMed Central PMCID: PMC2832463.
5. Rebelo-de-Andrade H, Pereira C, Giria M, Prudencio E, Brito MJ, Cale E, et al. Outbreak of acute respiratory infection among infants in Lisbon, Portugal, caused by human adenovirus serotype 3 and a new 7/3 recombinant strain. *Journal of clinical microbiology*. 2010; 48(4):1391–6. Epub 2010/02/12. doi: [10.1128/JCM.02019-09](https://doi.org/10.1128/JCM.02019-09) PMID: [20147640](https://pubmed.ncbi.nlm.nih.gov/20147640/); PubMed Central PMCID: PMC2849616.
6. Hough HS, Gong H, Kajon AE, Jones MS, Kuschner RA, Lyons A, et al. Genome sequences of human adenovirus 14 isolates from mild respiratory cases and a fatal pneumonia, isolated during 2006–2007 epidemics in North America. *Respiratory research*. 2010; 11:116. Epub 2010/08/27. doi: [10.1186/1465-9921-11-116](https://doi.org/10.1186/1465-9921-11-116) PMID: [20738863](https://pubmed.ncbi.nlm.nih.gov/20738863/); PubMed Central PMCID: PMC2933684.
7. Kolavic-Gray SA, Binn LN, Sanchez JL, Cersovsky SB, Polyak CS, Mitchell-Raymundo F, et al. Large epidemic of adenovirus type 4 infection among military trainees: epidemiological, clinical, and

- laboratory studies. *Clin Infect Dis*. 2002; 35(7):808–18. Epub 2002/09/14. doi:CID020396 [pii] doi: [10.1086/342573](https://doi.org/10.1086/342573) PMID: [12228817](https://pubmed.ncbi.nlm.nih.gov/12228817/).
8. Kajon AE, Wadell G. Sequence analysis of the E3 region and fiber gene of human adenovirus genome type 7h. *Virology*. 1996; 215(2):190–6. Epub 1996/01/15. doi: [10.1006/viro.1996.0022](https://doi.org/10.1006/viro.1996.0022) PMID: [8560766](https://pubmed.ncbi.nlm.nih.gov/8560766/).
 9. Yamamoto D, Okamoto M, Lupisan S, Suzuki A, Saito M, Tamaki R, et al. Impact of human adenovirus serotype 7 in hospitalized children with severe fatal pneumonia in the Philippines. *Japanese journal of infectious diseases*. 2014; 67(2):105–10. PMID: [24647252](https://pubmed.ncbi.nlm.nih.gov/24647252/).
 10. Lai CY, Lee CJ, Lu CY, Lee PI, Shao PL, Wu ET, et al. Adenovirus serotype 3 and 7 infection with acute respiratory failure in children in Taiwan, 2010–2011. *PloS one*. 2013; 8(1):e53614. doi: [10.1371/journal.pone.0053614](https://doi.org/10.1371/journal.pone.0053614) PMID: [23326469](https://pubmed.ncbi.nlm.nih.gov/23326469/); PubMed Central PMCID: PMC3542335.
 11. Tsai HP, Kuo PH, Liu CC, Wang JR. Respiratory viral infections among pediatric inpatients and outpatients in Taiwan from 1997 to 1999. *Journal of clinical microbiology*. 2001; 39(1):111–8. doi: [10.1128/JCM.39.1.111-118.2001](https://doi.org/10.1128/JCM.39.1.111-118.2001) PMID: [11136758](https://pubmed.ncbi.nlm.nih.gov/11136758/); PubMed Central PMCID: PMC87689.
 12. Chang SY, Lee CN, Lin PH, Huang HH, Chang LY, Ko W, et al. A community-derived outbreak of adenovirus type 3 in children in Taiwan between 2004 and 2005. *Journal of medical virology*. 2008; 80(1):102–12. Epub 2007/11/28. doi: [10.1002/jmv.21045](https://doi.org/10.1002/jmv.21045) PMID: [18041026](https://pubmed.ncbi.nlm.nih.gov/18041026/).
 13. Lin KH, Lin YC, Chen HL, Ke GM, Chiang CJ, Hwang KP, et al. A two decade survey of respiratory adenovirus in Taiwan: the reemergence of adenovirus types 7 and 4. *Journal of medical virology*. 2004; 73(2):274–9. Epub 2004/05/04. doi: [10.1002/jmv.20087](https://doi.org/10.1002/jmv.20087) PMID: [15122804](https://pubmed.ncbi.nlm.nih.gov/15122804/).
 14. Lin YC, Lin KH, Ke GM, Lee MS, Lin PC, Liu HF, et al. Secular trend of genome types of respiratory adenovirus type 3 during 1983–2005: a study from Taiwan. *Archives of virology*. 2010; 155(2):287–92. Epub 2009/12/25. doi: [10.1007/s00705-009-0575-6](https://doi.org/10.1007/s00705-009-0575-6) PMID: [20033743](https://pubmed.ncbi.nlm.nih.gov/20033743/).
 15. Lin KH, Chow TY, Sheu MM, Huang WL, Chen CW. A rapid and simple method for preparation of adenovirus DNA for restriction endonuclease cleavage studies. *The Kaohsiung journal of medical sciences*. 1986; 2(12):774–7. PMID: [2836602](https://pubmed.ncbi.nlm.nih.gov/2836602/).
 16. Saitoh-Inagawa W, Oshima A, Aoki K, Itoh N, Isobe K, Uchio E, et al. Rapid diagnosis of adenoviral conjunctivitis by PCR and restriction fragment length polymorphism analysis. *Journal of clinical microbiology*. 1996; 34(9):2113–6. PMID: [8862567](https://pubmed.ncbi.nlm.nih.gov/8862567/); PubMed Central PMCID: PMC229199.
 17. Li QG, Zheng QJ, Liu YH, Wadell G. Molecular epidemiology of adenovirus types 3 and 7 isolated from children with pneumonia in Beijing. *Journal of medical virology*. 1996; 49(3):170–7. Epub 1996/07/01. doi: [10.1002/\(SICI\)1096-9071\(199607\)49:3<170::AID-JMV3>3.0.CO;2-1](https://doi.org/10.1002/(SICI)1096-9071(199607)49:3<170::AID-JMV3>3.0.CO;2-1) PMID: [8818961](https://pubmed.ncbi.nlm.nih.gov/8818961/).
 18. Azar R, Varsano N, Mileguir F, Mendelson E. Molecular epidemiology of adenovirus type 7 in Israel: identification of two new genome types, Ad7k and Ad7d2. *Journal of medical virology*. 1998; 54(4):291–9. Epub 1998/04/29. PMID: [9557295](https://pubmed.ncbi.nlm.nih.gov/9557295/).
 19. Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S. MEGA5: Molecular Evolutionary Genetics Analysis Using Maximum Likelihood, Evolutionary Distance, and Maximum Parsimony Methods. *Molecular biology and evolution*. 2011; 28(10):2731–9. Epub 2011/05/07. doi:msr121 [pii] doi: [10.1093/molbev/msr121](https://doi.org/10.1093/molbev/msr121) PMID: [21546353](https://pubmed.ncbi.nlm.nih.gov/21546353/).
 20. Tamura K, Stecher G, Peterson D, Filipiński A, Kumar S. MEGA6: Molecular Evolutionary Genetics Analysis version 6.0. *Molecular biology and evolution*. 2013; 30(12):2725–9. doi: [10.1093/molbev/mst197](https://doi.org/10.1093/molbev/mst197) PMID: [24132122](https://pubmed.ncbi.nlm.nih.gov/24132122/); PubMed Central PMCID: PMC3840312.
 21. Schmidt HA, Strimmer K, Vingron M, von Haeseler A. TREE-PUZZLE: maximum likelihood phylogenetic analysis using quartets and parallel computing. *Bioinformatics*. 2002; 18(3):502–4. Epub 2002/04/06. PMID: [11934758](https://pubmed.ncbi.nlm.nih.gov/11934758/).
 22. Guindon S, Dufayard JF, Lefort V, Anisimova M, Hordijk W, Gascuel O. New algorithms and methods to estimate maximum-likelihood phylogenies: assessing the performance of PhyML 3.0. *Systematic biology*. 2010; 59(3):307–21. doi: [10.1093/sysbio/syq010](https://doi.org/10.1093/sysbio/syq010) PMID: [20525638](https://pubmed.ncbi.nlm.nih.gov/20525638/).
 23. Drummond AJ, Suchard MA, Xie D, Rambaut A. Bayesian phylogenetics with BEAUti and the BEAST 1.7. *Molecular biology and evolution*. 2012; 29(8):1969–73. Epub 2012/03/01. doi: [10.1093/molbev/mss075](https://doi.org/10.1093/molbev/mss075) PMID: [22367748](https://pubmed.ncbi.nlm.nih.gov/22367748/); PubMed Central PMCID: PMC3408070.
 24. Ayres DL, Darling A, Zwickl DJ, Beerli P, Holder MT, Lewis PO, et al. BEAGLE: an application programming interface and high-performance computing library for statistical phylogenetics. *Systematic biology*. 2012; 61(1):170–3. doi: [10.1093/sysbio/syr100](https://doi.org/10.1093/sysbio/syr100) PMID: [21963610](https://pubmed.ncbi.nlm.nih.gov/21963610/); PubMed Central PMCID: PMC3243739.
 25. Shapiro B, Rambaut A, Drummond AJ. Choosing appropriate substitution models for the phylogenetic analysis of protein-coding sequences. *Molecular biology and evolution*. 2006; 23(1):7–9. doi: [10.1093/molbev/msj021](https://doi.org/10.1093/molbev/msj021) PMID: [16177232](https://pubmed.ncbi.nlm.nih.gov/16177232/).
 26. Baele G, Lemey P, Bedford T, Rambaut A, Suchard MA, Alekseyenko AV. Improving the accuracy of demographic and molecular clock model comparison while accommodating phylogenetic uncertainty.

- Molecular biology and evolution. 2012; 29(9):2157–67. doi: [10.1093/molbev/mss084](https://doi.org/10.1093/molbev/mss084) PMID: [22403239](https://pubmed.ncbi.nlm.nih.gov/22403239/); PubMed Central PMCID: [PMC3424409](https://pubmed.ncbi.nlm.nih.gov/PMC3424409/).
27. Murrell B, Moola S, Mabona A, Weighill T, Sheward D, Kosakovsky Pond SL, et al. FUBAR: a fast, unconstrained bayesian approximation for inferring selection. *Molecular biology and evolution*. 2013; 30(5):196–205. doi: [10.1093/molbev/mst030](https://doi.org/10.1093/molbev/mst030) PMID: [23420840](https://pubmed.ncbi.nlm.nih.gov/23420840/); PubMed Central PMCID: [PMC3670733](https://pubmed.ncbi.nlm.nih.gov/PMC3670733/).
 28. Kosakovsky Pond SL, Poon AF, Leigh Brown AJ, Frost SD. A maximum likelihood method for detecting directional evolution in protein sequences and its application to influenza A virus. *Molecular biology and evolution*. 2008; 25(9):1809–24. doi: [10.1093/molbev/msn123](https://doi.org/10.1093/molbev/msn123) PMID: [18511426](https://pubmed.ncbi.nlm.nih.gov/18511426/); PubMed Central PMCID: [PMC2515872](https://pubmed.ncbi.nlm.nih.gov/PMC2515872/).
 29. Poon AF, Frost SD, Pond SL. Detecting signatures of selection from DNA sequences using Datamonkey. *Methods Mol Biol*. 2009; 537:163–83. Epub 2009/04/21. doi: [10.1007/978-1-59745-251-9_8](https://doi.org/10.1007/978-1-59745-251-9_8) PMID: [19378144](https://pubmed.ncbi.nlm.nih.gov/19378144/).
 30. Delport W, Poon AF, Frost SD, Kosakovsky Pond SL. Datamonkey 2010: a suite of phylogenetic analysis tools for evolutionary biology. *Bioinformatics*. 2010; 26(19):2455–7. Epub 2010/07/31. doi: [10.1093/bioinformatics/btq429](https://doi.org/10.1093/bioinformatics/btq429) PMID: [20671151](https://pubmed.ncbi.nlm.nih.gov/20671151/); PubMed Central PMCID: [PMC2944195](https://pubmed.ncbi.nlm.nih.gov/PMC2944195/).
 31. Tsou TP, Tan BF, Chang HY, Chen WC, Huang YP, Lai CY, et al. Community Outbreak of Adenovirus, Taiwan, 2011. *Emerging infectious diseases*. 2012; 18(11):1825–32. Epub 2012/10/25. doi: [10.3201/eid1811.120629](https://doi.org/10.3201/eid1811.120629) PMID: [23092602](https://pubmed.ncbi.nlm.nih.gov/23092602/).
 32. Kajon AE, Dickson LM, Metzgar D, Hough HS, Lee V, Tan BH. Outbreak of febrile respiratory illness associated with adenovirus 11a infection in a Singapore military training cAMP. *Journal of clinical microbiology*. 2010; 48(4):1438–41. Epub 2010/02/05. doi: [10.1128/JCM.01928-09](https://doi.org/10.1128/JCM.01928-09) PMID: [20129957](https://pubmed.ncbi.nlm.nih.gov/20129957/); PubMed Central PMCID: [PMC2849553](https://pubmed.ncbi.nlm.nih.gov/PMC2849553/).
 33. Darriba D, Taboada GL, Doallo R, Posada D. jModelTest 2: more models, new heuristics and parallel computing. *Nature methods*. 2012; 9(8):772. Epub 2012/08/01. doi: [10.1038/nmeth.2109](https://doi.org/10.1038/nmeth.2109) PMID: [22847109](https://pubmed.ncbi.nlm.nih.gov/22847109/).
 34. Ikeda Y, Yamaoka K, Noda M, Ogino T. Genome types of adenovirus type 7 isolated in Hiroshima City. *Journal of medical virology*. 2003; 69(2):215–9. Epub 2003/04/10. doi: [10.1002/jmv.10287](https://doi.org/10.1002/jmv.10287) PMID: [12683410](https://pubmed.ncbi.nlm.nih.gov/12683410/).
 35. Walsh MP, Chintakuntlawar A, Robinson CM, Madisch I, Harrach B, Hudson NR, et al. Evidence of molecular evolution driven by recombination events influencing tropism in a novel human adenovirus that causes epidemic keratoconjunctivitis. *PloS one*. 2009; 4(6):e5635. Epub 2009/06/06. doi: [10.1371/journal.pone.0005635](https://doi.org/10.1371/journal.pone.0005635) PMID: [19492050](https://pubmed.ncbi.nlm.nih.gov/19492050/); PubMed Central PMCID: [PMC2685984](https://pubmed.ncbi.nlm.nih.gov/PMC2685984/).
 36. Yusof MA, Rashid TR, Thayan R, Othman KA, Hasan NA, Adnan N, et al. Human adenovirus type 7 outbreak in Police Training Center, Malaysia, 2011. *Emerging infectious diseases*. 2012; 18(5):852–4. Epub 2012/04/21. doi: [10.3201/eid1805.110865](https://doi.org/10.3201/eid1805.110865) PMID: [22515984](https://pubmed.ncbi.nlm.nih.gov/22515984/); PubMed Central PMCID: [PMC3358062](https://pubmed.ncbi.nlm.nih.gov/PMC3358062/).
 37. Akiyoshi K, Suga T, Fukui K, Taniguchi K, Okabe N, Fujimoto T. Outbreak of epidemic keratoconjunctivitis caused by adenovirus type 54 in a nursery school in Kobe City, Japan in 2008. *Japanese journal of infectious diseases*. 2011; 64(4):353–5. Epub 2011/07/27. PMID: [21788718](https://pubmed.ncbi.nlm.nih.gov/21788718/).
 38. Xie L, Yu XF, Sun Z, Yang XH, Huang RJ, Wang J, et al. Two adenovirus serotype 3 outbreaks associated with febrile respiratory disease and pharyngoconjunctival fever in children under 15 years of age in Hangzhou, China, during 2011. *Journal of clinical microbiology*. 2012; 50(6):1879–88. Epub 2012/03/24. doi: [10.1128/JCM.06523-11](https://doi.org/10.1128/JCM.06523-11) PMID: [22442311](https://pubmed.ncbi.nlm.nih.gov/22442311/); PubMed Central PMCID: [PMC3372113](https://pubmed.ncbi.nlm.nih.gov/PMC3372113/).
 39. Lee WJ, Jung HD, Cheong HM, Kim K. Molecular epidemiology of a post-influenza pandemic outbreak of acute respiratory infections in Korea caused by human adenovirus type 3. *Journal of medical virology*. 2015; 87(1):10–7. Epub 2014/06/04. doi: [10.1002/jmv.23984](https://doi.org/10.1002/jmv.23984) PMID: [24889391](https://pubmed.ncbi.nlm.nih.gov/24889391/).
 40. Cheng CC, Huang LM, Kao CL, Lee PI, Chen JM, Lu CY, et al. Molecular and clinical characteristics of adenoviral infections in Taiwanese children in 2004–2005. *European journal of pediatrics*. 2008; 167(6):633–40. Epub 2007/09/19. doi: [10.1007/s00431-007-0562-4](https://doi.org/10.1007/s00431-007-0562-4) PMID: [17876605](https://pubmed.ncbi.nlm.nih.gov/17876605/).
 41. Tabain I, Ljubin-Sternak S, Cepin-Bogovic J, Markovinovic L, Knezovic I, Mlinaric-Galinovic G. Adenovirus respiratory infections in hospitalized children: clinical findings in relation to species and serotypes. *The Pediatric infectious disease journal*. 2012; 31(7):680–4. Epub 2012/04/21. doi: [10.1097/INF.0b013e318256605e](https://doi.org/10.1097/INF.0b013e318256605e) PMID: [22517338](https://pubmed.ncbi.nlm.nih.gov/22517338/).
 42. Tang L, Wang L, Tan X, Xu W. Adenovirus serotype 7 associated with a severe lower respiratory tract disease outbreak in infants in Shaanxi Province, China. *Virology journal*. 2011; 8:23. Epub 2011/01/19. doi: [10.1186/1743-422X-8-23](https://doi.org/10.1186/1743-422X-8-23) PMID: [21241515](https://pubmed.ncbi.nlm.nih.gov/21241515/); PubMed Central PMCID: [PMC3030507](https://pubmed.ncbi.nlm.nih.gov/PMC3030507/).
 43. Choi EH, Lee HJ, Kim SJ, Eun BW, Kim NH, Lee JA, et al. Ten-year analysis of adenovirus type 7 molecular epidemiology in Korea, 1995–2004: implication of fiber diversity. *J Clin Virol*. 2006; 35(4):388–93. Epub 2006/02/07. doi: [10.1016/j.jcv.2006.01.001](https://doi.org/10.1016/j.jcv.2006.01.001) PMID: [16458589](https://pubmed.ncbi.nlm.nih.gov/16458589/).

44. Biere B, Schweiger B. Human adenoviruses in respiratory infections: sequencing of the hexon hyper-variable region reveals high sequence variability. *J Clin Virol.* 2010; 47(4):366–71. Epub 2010/02/13. doi: [10.1016/j.jcv.2010.01.005](https://doi.org/10.1016/j.jcv.2010.01.005) PMID: [20149723](https://pubmed.ncbi.nlm.nih.gov/20149723/).
45. Choi EH, Kim HS, Park KH, Lee HJ. Genetic heterogeneity of the hexon gene of adenovirus type 3 over a 9-year period in Korea. *Journal of medical virology.* 2006; 78(3):379–83. Epub 2006/01/19. doi: [10.1002/jmv.20550](https://doi.org/10.1002/jmv.20550) PMID: [16419117](https://pubmed.ncbi.nlm.nih.gov/16419117/).
46. Li Q, Wadell G. Genetic variability of hexon loops 1 and 2 between seven genome types of adenovirus serotype 7. *Archives of virology.* 1999; 144(9):1739–49. Epub 1999/10/29. PMID: [10542023](https://pubmed.ncbi.nlm.nih.gov/10542023/).