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Clinical and molecular features of adenovirus type 2, 3, and 7 infections in children in an outbreak in Taiwan, 2011

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ABSTRACT

Objectives: We studied paediatric patients with human adenovirus (HAdV) infection during the 2011 outbreak in northern Taiwan to define the clinical features of different HAdV genotypes in children.
Methods: Between January and December 2011, 637 patients <19 years of age exhibited culture-confirmed adenoviral infection in Chang Gung Memorial Hospital, and provided specimens available for genotyping by multiplex real-time PCR. Clinical data were collected retrospectively.
Results: Excluding five cases with multiple genotypes, 632 cases were included for analysis. Three genotypes were identified, including HAdV-3 (429/632; 67.6%), HAdV-7 (144/632; 22.6%) and HAdV-2 (59/632; 9.8%). Median age was 4.58 years (range 2 months to 18 years), with children infected with HAdV-3 significantly older (82.9% >3 years; p <0.001). Of the 621 inpatients, 98.2% had fevers and all exhibited respiratory symptoms, 75 patients (12.1%) had lower respiratory tract infections, 20 (3.2%) required intensive care (HAdV-2: 1; HAdV-3: 8; and HAdV-7: 11), and three died (all HAdV-7-infected). HAdV-3-infected patients were significantly more likely to have upper respiratory symptoms and a high serum C-reactive protein level >100 mg/L, whereas leucocytosis (white blood cell count >15 000/mm^3) was more common in HAdV-2-infected patients (p 0.007). HAdV-7 infections were significantly associated with a longer duration of fever, leucopenia (white blood cell count <5000/mm^3), thrombocytopenia (platelet count <150 000/mm^3), lower respiratory tract infections, a longer length of hospital stay, and requiring intensive care (all p <0.001).
Conclusion: Childhood HAdV-2, HAdV-3 and HAdV-7 infections may exhibit different clinical manifestations. Although HAdV-3 was the most prevalent genotype observed during the 2011 Taiwan outbreak, HAdV-7 caused more severe disease characteristics and outcomes. **M.-R. Lin, CMI 2017;23:110**

Introduction

Human adenovirus (HAdV) is a common pathogen in children that causes a variety of diseases, including respiratory disease, gastroenteritis, conjunctivitis and haemorrhagic cystitis [1–6]. HAdV is a double-stranded DNA virus that can be subdivided into >60 genotypes in seven species classified from A to G based on genomic homology [7]. Each genotype can cause different clinical manifestations, with genotypes 2, 3, 4, 7, 14, 21 and 55 resulting in more severe and disseminated diseases, including pneumonia and encephalitis [8–14].

According to previous studies of adenoviral epidemiology in Taiwan, HAdV-3 has circulated annually since 1999 and remains the dominant circulating genotype [15] as compared with the low prevalence of HAdV-7. A large community outbreak of adenoviral infections was detected by the nationwide surveillance system of the Centre for Disease Control-Taiwan (CDC-Taiwan) between weeks 11 and 41 in 2011 [16], with the average HAdV-positivity rate increasing from 5.75% between 2008 and 2010 to 25.9% in 2011, along with an increase in the number of children admitted to the intensive care

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units of two medical centres in northern Taiwan owing to HAdV infection [17]. During this period, HAdV-7 re-emergence also occurred, with HAdV-7 proportions in Taiwan increasing significantly from 0.3% between 2008 and 2010 to 10% in 2011 [16].

Studies showed that HAdV-7 is associated with more severe clinical courses and complications [18–21]; however, there are no reports concerning the clinical features associated with large outbreaks of HAdV-7 infections in children. To better understand the clinical features and complications of different HAdV infections in children, we conducted a retrospective study to investigate the clinical features of children with HAdV infections during the 2011 outbreak in Taiwan.

Materials and methods

Study design and population

We conducted a retrospective study of children with adenoviral infections in Chang Gung Memorial Hospital during 2011. Children <19 years old with culture-confirmed adenoviral infections were enrolled, excluding those infected with more than two types of virus or with insufficient samples for further genotype stratification. Demographic data were collected and analysed from all patients, whereas clinical symptoms, laboratory results, images, complications and clinical outcomes were collected and analysed from inpatients only.

Ethics statement

This retrospective study was approved by the Institutional Review Board of Chang Gung Memorial Hospital (CGMH; No. 100-2518B). Clinical information was analysed anonymously so informed consent was waived.

Viral isolation, typing and sequencing

Procedures used for viral isolation and identification and designs of multiplex real-time PCR for HAdV genotyping are described in the Supplementary material (Data S1). HAdV sequencing was performed on full-length hexon genes as previously described [22]. PCR amplification was performed using specific primers (see Supplementary material, Table S1).

Definitions

We defined cases with lower respiratory tract infection (LRTI) as those having at least a pneumonia patch according to chest plain films or those without a pneumonia patch but with severe respiratory distress requiring oxygen supplementation and intensive care. In Taiwan, *Mycoplasma pneumoniae* caused frequent LRTIs in children >3 years old, and is usually screened for in this age group when pneumonia is suspected. Despite possible positivity for urinary pneumococcal antigen owing to asymptomatic carriage, some paediatricians still preferred using it as a screening tool, because *Streptococcus pneumoniae* is a common pneumonia pathogen in children <5 years old. For possible *M. pneumoniae* co-infection, we considered three situations as positive results (summarized in the Supplementary material, Data S1). Clinical laboratory data on admission and peak or nadir values during hospitalization were collected. White blood cell count >15 000/μL was defined as leucocytosis but a count of <5000/μL was defined as leucopenia. Platelet count >450 000/μL was defined as thrombocytosis and <150 000/μL as thrombocytopenia. Serum creatinine levels >1.0 mg/dL were considered evidence of impaired renal function, whereas a three-fold elevation in normal aspartate aminotransferase and alanine aminotransferase levels was considered evidence of impaired liver function.

Molecular phylogenetic analysis using the maximum likelihood method

All 107 complete hexon sequences were downloaded from GenBank and aligned using ClustalO [23] against 25 sequences provided in this study. The evolutionary history of these sequences was inferred using the maximum likelihood method based on the Hasegawa–Kishino–Yano model [24]. Evolutionary analyses were conducted using MEGA7 [25] and evaluated using 1000 bootstrap replicates.

Statistical analysis

We used the chi-square test or Fisher's exact test to compare categorical variables. Non-categorical variables were compared by one-way independent analysis of variance with post-hoc analysis. We looked for factors associated with LRTI and intensive care admission, using a multivariate logistic regression analysis (variables with p values <0.20 in univariate analysis were entered in the model). Statistical significance was set at $p < 0.05$.

Results

Of 1598 children with positive virus cultures, adenovirus alone was identified in 661 specimens (41.4%). Other viruses identified are listed in the Supplementary material (Table S2). Further genotyping by PCR was possible in 637 patients who had adequate specimens, most of which were obtained by throat swab (616/632; 97.5%; see Supplementary material, Table S3). After excluding five cases with multiple genotypes, a total of 632 cases were included for further analysis. Only three genotypes were identified, with HAdV-3 (429/632; 67.6%) being the most common, followed by HAdV-7 (144/632; 22.6%) and HAdV-2 (59/632; 9.8%). Of the 621 patients (98.3%) hospitalized, 75 (12%) had defined LRTIs, 20 (3.2%) required intensive care, and three (0.5%) eventually died (Fig. 1).

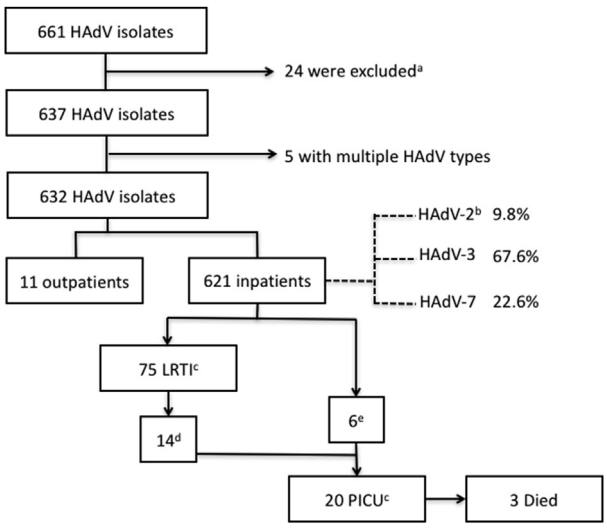

Fig. 1. Flow chart describing case selection. [a] Patients excluded due to insufficient samples. [b] HAdV-2, -3 and -7, human adenovirus type 2, 3, and 7. [c] LRTI, lower respiratory tract infection; PICU, paediatric intensive care unit. [d] LRTI patients (14/75) exhibiting severe respiratory distress. [e] These patients included three with neurological dysfunction, two suffering from shock, and one with massive bleeding in the gastrointestinal tract.

Demographic and clinical characteristics

The median age was 4.58 years (range: 2 months to 18 years), with HAdV-3-infected children being significantly older than children harbouring other infections (Table 1). Detailed clinical characteristics of hospitalized children are shown in Table 1. HAdV-3-infected children were more likely to experience symptoms of upper respiratory tract infection, including cough, rhinorrhoea, nasal congestion and exudative tonsillitis. HAdV-7-infected children experienced longer duration of fever and were more likely to be less active. Skin rash accompanied with oral ulcers (6.9%) mimicking clinical symptoms of enterovirus infection was also observed, particularly in HAdV-7-infected patients. All clinical diagnoses are listed in the Supplementary material (Table S4).

Laboratory parameters

Detailed laboratory data are shown in Table 2. Leucocytosis was noted in 163 (26.2%) children, and a serum C-reactive protein (CRP) level ≥40 mg/L was identified in 423 (68.1%) children. HAdV-2-infected children showed leucocytosis and thrombocytosis, whereas HAdV-3-infected children were characterized by high serum CRP levels. By contrast, HAdV-7-infected children were significantly more likely to show leucopenia, thrombocytopenia, and impaired liver and renal function.

LRT involvement and radiological findings

LRTI was diagnosed in 75 cases, including 63 with a pneumonia patch observed on chest plain films and 12 with respiratory distress

Table 1
Demographic data and clinical characteristics of 621 hospitalized children infected with different HAdV genotypes

Characteristics	HAdV-2, *n* (%)	HAdV-3, *n* (%)	HAdV-7, *n* (%)	p[a]
Case number	58	422	141	
Male	33 (56.9)	237 (56.1)	89 (63.1)	0.346
Age, mean ± SD (months)	38.90±23.01	61.59±30.54	57.98±46.52	<0.001
>3 years	31 (53.4)	350 (82.9)	84 (59.6)	<0.001
General symptoms				
Fever	54 (93.1)	417 (98.8)	138 (97.9)	0.012
Fever >39°C	51 (87.9)	396 (93.8)	133 (94.3)	0.209
Duration, mean ± SD (days)	5.33±3.03	5.39±2.13	6.58±2.90	<0.001
>7 days	17 (29.3)	36 (8.5)	40 (28.4)	<0.001
Decreased appetite	49 (84.5)	352 (83.4)	128 (90.8)	0.102
Decreased activity	33 (56.9)	282 (66.8)	116 (82.3)	<0.001
Respiratory symptoms				
Cough	47 (81.0)	362 (85.8)	106 (75.2)	0.014
Rhinorrhoea	40 (69.0)	306 (72.5)	79 (56.0)	0.007
Nasal congestion	29 (50.0)	234 (55.5)	53 (37.6)	0.001
Sore throat	17 (29.3)	189 (44.8)	58 (41.1)	0.077
Exudate coating	21 (36.2)	202 (47.9)	47 (33.9)	0.005
Oral ulcers	1 (1.7)	24 (5.7)	18 (12.8)	0.004
Conjunctivitis	9 (15.5)	79 (18.7)	24 (17.0)	0.786
Extrapulmonary symptoms				
Skin rash	4 (6.9)	22 (5.2)	17 (12.1)	0.021
Headache	4 (6.9)	51 (12.1)	11 (7.9)	0.232
Abdominal pain	12 (20.7)	124 (29.4)	38 (27.0)	0.365
Vomiting	13 (22.4)	119 (28.2)	41 (29.1)	0.611
Diarrhoea	10 (17.2)	117 (27.7)	57 (27.0)	0.365
Underlying diseases	1 (1.7)	10 (2.4)	3 (2.1)	0.947
Neurology	0	2	1	
Pulmonary	0	1	0	
Gastrointestinal	0	1	2	
Cardiovascular	0	0	0	
Others	1	6	0	
Complication				
LRTI	3 (3.4)	40 (9.0)	32 (20.6)	<0.001
Respiratory distress	1 (1.7)	13 (3.1)	8 (5.7)	0.259
Intubation	1 (1.7)	3 (0.7)	3 (2.1)	0.333
Duration (days)	2	6.67±0.58	25.33±32.62	0.017
Pneumonia patch	3 (6.9)	32 (8.5)	28 (19.9)	<0.001
Single lobe	100%	66%	61%	
Multiple lobes	0%	34%	39%	
Pleural effusion	0	3 (0.7)	8 (5.7)	<0.001
PICU	1 (1.7)	8 (1.9)	11 (7.8)	<0.001
Duration (days)	4	6.38±2.72	11.45±18.93	0.114
Mortality	0	0	3	
ECMO use	0	0	2	
Antibiotics treatment	30 (51.7)	275 (65.2)	76 (53.9)	<0.001
Co-infection with *Mycoplasma pneumoniae*	0	5 (1.2)	4 (2.8)	0.305
Co-infection with pneumococcus	0	0	4 (2.8)	0.15
LOS (days)	3.84±1.25	4.40±3.69	6.18±7.32	<0.001

Abbreviations: ECMO, extracorporeal membrane oxygenation; HAdV, human adenovirus; LOS, length of hospital stay; LRTI, lower respiratory tract infection; PICU, paediatric intensive care unit.
[a] Statistical significance: p <0.05 via post hoc analysis.

Table 2
Laboratory findings from 621 hospitalized children infected with different HAdV genotypes

Laboratory data	HAdV-2, *n* (%)	HAdV-3, *n* (%)	HAdV-7, *n* (%)	p
Case number	58	422	141	
Haemogram				
WBC count (1000/μL)[a]	15.36±5.85	12.93±5.48	9.39±4.69	<0.001
Peak or nadir[b] WBC count	15.80±5.74	13.19±5.55	9.92±5.00	<0.001
<5000/μL	0	7 (1.7)	17 (12.1)	<0.001
>15 000/μL	28 (48.3)	117 (27.7)	18 (12.7)	<0.001
Hb, mean±SD (g/dl)	13.51±5.63	9.92±5.00	12.69±5.69	0.126
Platelet, mean±SD (1000/μL)	297.74±101.05	249.58±72.74	213.74±76.21	<0.001
<150 000/μL	0	12 (2.8)	22 (15.6)	<0.001
>450 000/μL	4 (6.9)	8 (1.9)	1 (0.7)	<0.001
Biochemistry				
CRP (mg/L)[a]	42.87±37.07	77.81±56.39	38.46±37.86	<0.001
Peak CRP (mg/L)[b]	46.75±38.97	84.47±55.15	44.83±39.76	<0.001
<40 mg/L	30 (51.7)	93 (22.0)	75 (53.2)	<0.001
>100 mg/L	7 (12.1)	153 (36.3)	14 (9.9)	<0.001
BUN, mean±SD (mg/dL)	8.99±3.54	7.84±3.55	8.52±5.78	0.463
Cr, mean±SD (mg/dL)	0.33±0.10	0.37±0.11	0.62±1.79	0.146
>1.0 mg/dL	2/24 (8.3)	5/176 (2.8)	7/66 (10.6)	0.043
AST (U/L)	50.91±79.06	41.02±66.22	57.97±57.66	0.104
>3-fold of normal value	0/33	0/250	3/88 (3.4)	0.008

Abbreviations: AST, aspartate aminotransferase; BUN, blood urea nitrogen; Cr, creatinine; CRP, C-reactive protein; HAdV, human adenovirus; Hb, haemoglobin; WBC, white blood cell;.
Note: Haemogram and CRP were checked in all 621 cases. Cr was checked in 266 cases (HAdV-2: 24 cases; HAdDV-3: 176 cases; and HAdV-7: 66 cases) and AST was checked in 371 cases (HAdV-2: 24 cases; HAdDV-3: 176 cases; and HAdV-7: 66 cases).
[a] First laboratory examination on admission.
[b] The highest or lowest laboratory value during hospitalization.

requiring both oxygen supplementation and intensive care, but without observation of a pneumonia patch. Most of these patients (91%) were previously healthy. Among the 63 pneumonia cases, most (65%) involved a single lobe and 11 (17%) exhibited pleural effusion. HAdV-7-infected patients were more likely to develop LRTI. Seven (9.3%) of the 75 LRTI cases eventually developed respiratory failure and required intubation, with three cases (43%) having underlying diseases. HAdV-7-infected patients also exhibited longer durations of intubation (Table 1). Compared with patients without LRTIs, those with LRTIs were more likely to show longer fever duration, leucopenia, and longer hospital stays and antibiotics treatment (Table 3). In multivariate analysis, underlying diseases, fever >7 days, leucopenia, antibiotic prescription and HAdV-7 infection were all independently associated with the risk of LRTI (see Supplementary material, Table S5). All 75 patients with LRTI had blood samples sent for bacterial culture and three samples yielded positive results (all coagulase-negative staphylococcus). Urine samples were collected for 38 cases. Bacterial cultures showed only one positive result, which was for *Escherichia coli* in the HAdV-7 group. Thirty-six cases were tested for *M. pneumoniae* co-infection, with most (63.9%) aged >3 years. Positive tests were obtained for 9 of 36 (25%) cases, including a positive PCR result in one patient. Among the 46 cases screened for pneumococcus, 63% were <5 years old. Four (8.7%) of 46 cases exhibited a positive result for urinary pneumococcal antigen. Sputum specimens from 18 cases underwent bacterial culture, with two yielding results indicating methicillin-resistant *Staphylococcus aureus* (on days 18 and 33 following admission, respectively). Pleural fluid specimens from two cases underwent microbiological testing, with both specimens subsequently positive for HAdV-7 according to virus culture, but negative for bacteria. Co-infections with *M. pneumoniae* or *S. pneumoniae* were not statistically different among patients infected with HAdV-2, -3, or -7.

Complications and clinical outcomes

Twenty (3.2%) patients were admitted to the intensive care unit, including 14 with respiratory distress, three with shock, and one each with acute encephalitis and status epilepticus with hydrocephalus, respectively. Among these, six (33%) had underlying chronic systemic diseases, including neurological diseases (cerebral palsy) for three cases, tracheo-oesophageal fistula for two, and

Table 3
Comparison of clinical features between 621 hospitalized children with or without LRTIs or PICU admission

Characteristics	LRTI, *n* (%)	Non-LRTI, *n* (%)	p	PICU, *n* (%)	Non-PICU, *n* (%)	p
Case number	75	546		20	601	
Genotypes						
HAdV-2	3 (4.0)	55 (10.1)	0.09	2 (10.0)	56 (9.3)	0.918
HAdV-3	40 (53.3)	382 (69.9)	0.004	8 (40.0)	414 (68.9)	0.006
HAdV-7	32 (42.7)	109 (19.9)	<0.001	10 (50.0)	131 (21.8)	0.003
Fever	74 (98.6)	535 (98.0)	0.757	19 (95.0)	590 (98.2)	0.311
Fever>39°C	72 (96.0)	508 (93.0)	0.331	18 (90.0)	561 (93.5)	0.536
Duration (days)	7.96±7.46	4.20±3.37	<0.001	8.7±5.31	5.55±2.26	<0.001
>7 days	36 (48.0)	57 (10.4)	<0.001	11 (55)	82 (13.6)	<0.001
Peak or nadir leucocyte count, mean±SD	12.99±7.46	12.68±5.47	0.673	15.72±8.13	12.59±5.58	0.004
<5000/μL	8 (10.6)	16 (2.9)	<0.001	2 (10.0)	22 (3.7)	0.148
>15 000/μL	22 (29.3)	141 (25.8)	0.227	9 (45.0)	154 (25.6)	0.053
Peak CRP, mean±SD (mg/L)	85.05±38.97	70.37±52.8	0.033	109.57±86.44	70.76±52.03	0.021
>100 mg/L	28 (37.7)	146 (26.8)	0.055	8 (40.0)	166 (27.6)	0.225
Underlying diseases	6 (8)	8 (1.5)	0.003	6 (30)	8 (1.3)	<0.001
Neurology	3	0		3	0	
Pulmonary	0	1		0	1	
Gastrointestinal	3	0		3	0	
Cardiovascular	0	7		0	7	
Others	1	6		1	6	
Antibiotic treatment	72 (96.0)	309 (56.6)	<0.001	20 (100)	361 (60.1)	<0.001
LOS (days)	9.13±9.42	4.20±3.37	<0.001	20.0±17.89	4.24±2.17	<0.001

Abbreviations: CRP, C-reactive protein; HAdV-2, -3 and -7, human adenoviruses types 2, 3 and 7; LOS, length of hospital stay; LRTI, lower respiratory tract infection; PICU, paediatric intensive care unit.

cystic fibrosis for one. These 20 patients experienced a longer duration of fever and length of hospital stay, and were more likely to have pneumonia involving multiple lobes (61.5% versus 25.5%; p 0.012) and receive antibiotics treatment (100% versus 60.1%; p <0.001) (Table 3). Compared with those infected with non-HAdV-7 HAdV, patients infected with HAdV-7 were more likely to require intensive care (OR 4.43, 95% CI 1.78–10.91) and have a longer hospital stay. Three HAdV-7-infected patients eventually died, and extracorporeal-membrane oxygenation therapy was performed on two of these three patients. These two cases exhibited an underlying disease of cerebral palsy. Multivariate regression analysis revealed that underlying diseases, fever duration >7 days and HAdV-7 infection were predictive risk factors for severe infection requiring intensive care (see Supplementary material, Table S5).

Phylogenetic tree of the hexon gene

Because HAdV-7 caused more severe clinical symptoms, two, one and 22 hexon sequences from HAdV-7 isolated in 2002, 2004 and 2011, respectively, were analysed in this study. The 22 sequences isolated in 2011 were identical, but contained four substitutions at nucleotide positions 771, 1286, 1319 and 1785 not present in the other three sequences from 2002 and 2004. These substitutions included two synonymous (F257 and I595) and two non-synonymous (S429Y and Q440L) amino acid mutations. To compare these 22 strains with 82 others available in GenBank, a phylogenetic tree (Fig. 2) was constructed, revealing that these strains were grouped with and identical to 22 other strains, including Taiwanese strains isolated in 2011, Chinese strains isolated in 2009 and 2015, a Russian strain isolated in 2014 (Accession no. KU361344), and a strain from the USA isolated in 2014 (Accession no. KT963081). The three sequences from 2002 and 2004 were identical to a strain isolated in the USA (Accession no. AY601634) in 1997. These findings indicated that the hexon gene sequences isolated from HAdV-7-infected Taiwanese children were highly conserved.

Discussion

HAdV-2, -3 and -7 were the main causative agents of HAdV infections in the 2011 outbreak in Taiwan. Although HAdV-3 was the dominant circulating genotype, HAdV-7 caused more severe clinical symptoms, and HAdV-7-infected children were more likely to exhibit lower respiratory complications and higher mortality rates, especially in those with underlying neurological diseases.

HAdV is a common pathogen associated with acute respiratory infection in children. Previous epidemiological and molecular studies [26,27] indicated that respiratory syncytial virus was the most common pathogen in paediatric inpatients with acute respiratory infection and, compared with respiratory syncytial virus, human rhinovirus and bocavirus, they also found that HAdV tended to infect older children and was more likely to cause leucocytosis, high CRP levels, longer hospital stays, pneumonia and more frequent antibiotics prescriptions [26]. Among our studied cohort, 41.4% of paediatric patients with acute respiratory infections exhibited HAdV infection, with ~3.4% exhibiting co-infection identified by viral culture instead of real-time PCR. This might explain the underestimated rate of co-infection; however, our findings might reflect a community outbreak of HAdV, whereas most cases admitted to the hospital were due to more severe clinical presentations. Notably, none of our cases involved a diagnosis of bronchiolitis. This finding might be explained by 75% of the patients being >3 years old, as well as diagnoses by clinical physicians of bacterial infection rather than viral infection due to leucocytosis and high CRP levels.

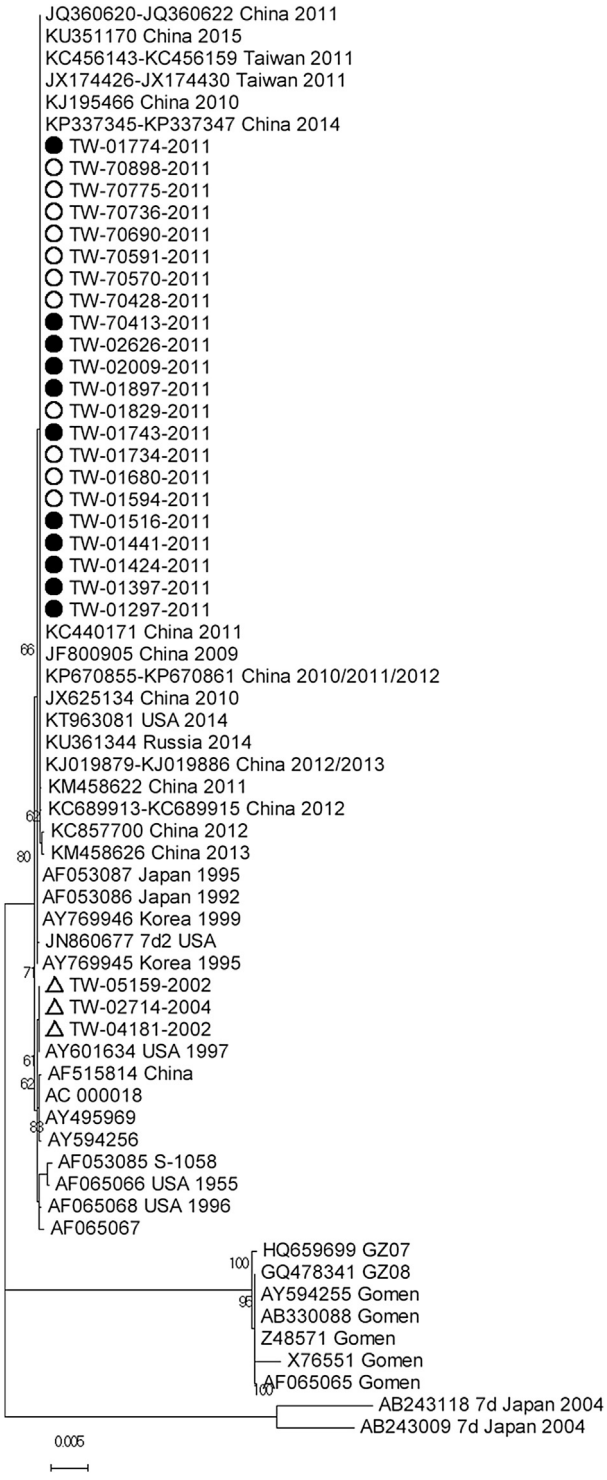

Fig. 2. Phylogenetic tree for human adenovirus type 7 (HAdV-7) hexon genes. This phylogenetic tree was derived from the HAdV-7 hexon gene and evaluated using 1000 bootstrap replicates. The 25 sequences provided in this study are labelled by their strain names. Among these, 11 severe and 11 non-severe cases are marked with black and white circles, respectively, and three cases isolated in 2002 and 2004 are marked with triangles. Other sequences downloaded from GenBank are labelled by either accession number, location and year or by strain name.

HAdV-7 was involved in a community outbreak in southern Taiwan from November 1999 to March 2000 [15]. However, over the previous decade, no HAdV-7 outbreaks and only a few HAdV-7 cases during 2008 and 2010 were observed according to

surveillance data from CDC-Taiwan [16]. Studies indicated that HAdV-7 was more likely to cause lower respiratory tract involvement and lead to long-term pulmonary sequelae compared with HAdV-3 [28]. This study revealed HAdV-7 re-emergence during the 2011 community outbreak in northern Taiwan.

Compared with those infected with HAdV-2 or HAdV-3, HAdV-7-infected children experienced longer durations of fever and hospital stay and were significantly associated with leucopenia, thrombocytopenia, and impaired liver and renal function. By contrast, HAdV-3 usually leads to leucocytosis and a high serum CRP level [29,30].

A LRTI was the most common complication associated with HAdV infection during this outbreak. Compared with HAdV-2 and HAdV-3, HAdV-7 was significantly associated with pulmonary complications and intensive care requirements, even in otherwise healthy children. Underlying medical problems, especially neurological diseases, were important risk factors associated with development of respiratory failure and subsequent higher mortality rates. Life-threatening complications were also reported in children with disabilities during an HAdV-7 outbreak in a residential facility for severely disabled children [31]. Lai *et al.* [17] also reported a total of 45 paediatric patients with HAdV infection that required intensive care in two medical centres, including our hospital, in northern Taiwan in 2010–2011 (16 patients overlapped with this study). Among these, HAdV-7 was the main causative agent (49%) and resulted in higher mortality rates, particularly in those with underlying neurological diseases. Pneumonia was also the most common clinical diagnosis (89%), with half of these patients subsequently developing respiratory failure. A previous study suggested that HAdV-7 is strongly related to severe infections, pneumonia and underlying neurological diseases and is a risk factor for severe HAdV infection [32].

During the 2011 outbreak, HAdV-3 remained the dominant circulating genotype in Taiwan, with >80% of HAdV-3-infected children >3 years old. These children were significantly more likely to manifest upper respiratory tract symptoms. A higher serum CRP level (>40 mg/L) was also noted in nearly 80% of HAdV-3-infected children and mimicked bacterial infection, subsequently resulting in antibiotics prescription for two-thirds of the patients. These findings were consistent with those reported for the 2004 and 2005 HAdV-3 outbreaks in Taiwan [29].

HAdV-7 hexon sequences [25] from Taiwanese patients with clinical symptoms of varying severity were nearly identical with other Taiwanese strains from 2011 and Chinese strains from 2009 and 2015 (Fig. 2). Although one of those Chinese strains caused an outbreak of severe lower respiratory tract disease [13], hexon sequences from 2011 cases were highly conserved [18] and contained only two non-synonymous mutations (S429Y and Q440L) as compared with the Taiwanese strains from 2002 and 2004.

This study has several limitations. First, as a retrospective study, not all clinical information and laboratory data could be collected. Second, nearly all cases (98%) in this study were inpatients, which might result in overestimation of the proportion of HAdV-7 infection and disease severity. Third, co-infection rates might have been underestimated. Virus isolation by tissue culture was used in this study; however, viruses, including human bocavirus, coronavirus and human metapneumovirus, cannot present cytopathogenic effects in selected cells.

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Transparency declaration

The authors report no conflicts of interest.

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Appendix A. Supporting information

Additional Supporting Information may be found in the online version of this article at http://dx.doi.org/10.1016/j.cmi.2016.11.004.

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