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Original Article

Molecular epidemiology and clinical features of adenovirus infection in Taiwanese children, 2014

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Abstract *Background/purposes:* Human adenovirus (HAdV) infection is prevalent and has an important clinical impact on children. We aim to investigate the molecular epidemiology of HAdV infection and discover the correlations between clinical features and HAdV species in an HAdV outbreak of 2014.
Methods: This is a retrospective study, enrolling patients under 19 years of age with HAdV infection at the National Taiwan University Hospital in 2014. We gathered the demographic and clinical data, carried out molecular typing and constructed a phylogenetic tree. Statistical analyses were performed in terms of HAdV species and hospitalization.
Results: A total of 531 patients with HAdV infection were identified. HAdV-B accounted for the largest proportion ($n = 387$, 73%). On average, patients infected with HAdV-E were oldest, whereas those with HAdV-C infection were youngest ($p < 0.001$). Patients with HAdV-B (HAdV-3) infection were associated with a lower incidence of co-infection with other viruses ($p < 0.001$). Complications occurred in 203 (38%) patients. There were 149 (28%) patients requiring hospitalization. The risk factors for hospitalization included underlying neurological abnormalities, prematurity and the diagnosis of pneumonia. Five patients (1%) had severe HAdV infection requiring intensive care; all of them fully recovered. The phylogenetic study showed that the partial hexon genes of HAdV-1, HAdV-3, HAdV-4 and HAdV-5 remain stable over time.

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Conclusion: We established the molecular epidemiology of HAdV infection and demonstrated the relationship between clinical features and HAdV species.

Introduction

Infection with human adenovirus (HAdV) is prevalent in children. It frequently causes respiratory infections and, sometimes, ocular infections, gastroenteritis or cystitis. In some rare cases, it leads to myocarditis, hepatitis or encephalitis. In our previous two-year surveillance study on kindergarten children, we found that HAdV circulates throughout the year with a very high attack rate (about one per person—year).[1] In another prospective study on lower respiratory tract infection (LRI) among children under five years of age, HAdV is one of the most common pathogens.[2] Therefore, HAdV is highly transmissible and has an important clinical impact on young children.

HAdVs are classified into seven species, from *Human adenovirus A* (HAdV-A) to HAdV-G. Each species comprises several types. There are more than 60 HAdV types that have been identified.[3] HAdV-B (mainly HAdV types 3 and 7), HAdV-C (mainly HAdV types 1, 2, 5 and 6) and HAdV-E (mainly HAdV type 4) are the most frequent species associated with epidemics of acute respiratory infections in children.[4] The clinical severity of the different HAdV types varies, and the transmissibility may also change. For example, HAdV type 3 (HAdV-3), HAdV-4, HAdV-7, HAdV-14 and HAdV-21 are more likely to lead to severe infections. In addition, our previous study demonstrated that HAdV-3 seems to have a higher familial transmission rate.[5]

There were several community outbreaks of HAdV infection in Taiwan in recent decades. HAdV-7 and HAdV-3 were the major types in an outbreak during 1999—2000, whereas HAdV-4 was most prevailing in another two outbreaks in 2000 and 2001.[6] A community outbreak of HAdV-3 was recorded in northern Taiwan during 2004—2005, while a single outbreak of re-emergent HAdV-4 was noted in southern Taiwan in 2007.[7,8] There was a further nationwide outbreak of HAdV infection throughout Taiwan in 2011, which was primarily caused by dominant HAdV-3 and re-emergent HAdV-7.[9]

According to the nationwide viral surveillance system of the Taiwan Centers for Disease Control,[10] the percentage of HAdV isolated among all respiratory specimens collected from patients with influenza-like illness was usually below 5% during 2012—2013. The HAdV-positive rate, however, significantly increased in 2014 (with a rise of over 10% from April onwards), indicating a HAdV outbreak.[11] During the HAdV outbreak of 2014, HAdV was also the most common virus isolated, constituting 50% of all viruses from respiratory specimens.[12] Nevertheless, there has not been much data, in terms of molecular epidemiology and clinical features, of the 2014 HAdV outbreak. Therefore, we aim to establish the molecular epidemiology of HAdV infection and the correlations between clinical features and different HAdV species during the 2014 outbreak. This study was also taken with a view to identifying risk factors for hospitalization.

Methods

Patients and study design

We enrolled both inpatients and outpatients under 19 years of age with laboratory-confirmed HAdV infection at the National Taiwan University Hospital (a university-affiliated tertiary medical center) from January 1 to December 31, 2014. Laboratory-confirmed HAdV infection was defined as the isolation of HAdV and/or positive polymerase chain reaction (PCR) for HAdV. Demographic data, clinical features, diagnosis, hospitalization, intensive care requirement, complications and sequelae were collected and analyzed.

The Institutional Review Board of the National Taiwan University Hospital approved this study (No. 201405027RINA). Because it was a retrospective study without intervention and all data was analyzed anonymously, informed consent was waived.

Definitions

We defined complicated upper respiratory tract infection (URI) as acute sinusitis and/or acute otitis media. The criterion for diagnosis of co-infection with *Mycoplasma pneumoniae* was a positive PCR result for *M. pneumoniae* plus symptoms and signs associated with mycoplasmal infection (e.g., fever, cough, and infiltration or patch on chest radiograph). We defined co-infection with *Streptococcus pneumoniae* as isolation of *S. pneumoniae* from clinical specimens and/or the presence of *S. pneumoniae* antigen in urine or pleural fluid with clinical significance. Patients with complications were defined as those with complicated URI, LRI, encephalitis or intussusception. Secondary bacterial infection was defined as co-infection with *M. pneumoniae* or *S. pneumoniae* or other clinically significant and relevant bacterial infection with microbiological evidence (e.g. *Pseudomonas aeruginosa* pneumonia). We defined respiratory failure as the need of mechanical ventilation. Prematurity was defined as delivery before 37 weeks' gestation. Patients having laboratory-confirmed HAdV infection and requiring intensive care were classified as severe HAdV infection.

Virus isolation, molecular diagnosis and typing of adenovirus

(1) Virus isolation

Clinical specimens, primarily throat swabs, but also including nasopharyngeal swabs, rectal swabs, conjunctival swabs, urine and cerebrospinal fluid, were inoculated into human embryonic lung fibroblast (HEF), human laryngeal carcinoma (HEp-2), rhabdomyosarcoma (RD), monkey kidney (MK-2) and Madin—Darby canine kidney (MDCK) cell lines. When cytopathic effects developed, direct immunofluorescence assay was performed to identify the virus by virus-specific monoclonal antibodies.

(2) Molecular diagnosis of adenovirus

Total nucleic acids were extracted from clinical samples by using the MagNA Pure LC Total Nucleic Acid Isolation Kit (Roche, Mannheim, Baden-Württemberg, Germany). Pan-adenovirus real-time PCR was performed using the Light-Cycler (Roche, Mannheim, Baden-Württemberg, Germany) with adenovirus-specific primers, 5′-GCCACGGTGGGGTTTC-TAAACTT-3′ (Adenoquant 1, AQ1) and 5′-GCCCCAGTGGTCT-TACATGCACATC-3′ (Adenoquant 2, AQ2). The PCR probe was FAM 5′-TGCACCAGACCCGGGCTCAGGTACTCCGA-3′ TAMRA (Adenoprobe, AP), and was labelled on the ends with FAM and TAMRA as a fluorescent dye and a fluorescence quencher dye, respectively.[1,13]

(3) Molecular typing of adenovirus

Studies have demonstrated that a partial nucleotide sequence of the conserved region of the hexon gene can be used to differentiate different HAdV types.[14—16] Therefore, we performed nested PCRs using primers targeting the 3′ end of the conserved region of the hexon gene for the molecular typing of HAdV.[1,9] The first PCR was carried out using 3 μL of extracted HAdV DNA from clinical samples. The PCR primers were 5′-TACAACATYGGCTACCAGGG-3′ (positions 21,205 to 21,224) and 5′-GAGAASGGBGTRCG-SAGGTA-3′ (positions 21,722 to 21,703). The nested PCR was then performed with the primers of 5′-AACTTCCAGCCYAT-GAG-3′ (positions 21,274 to 21,290) and 5′-GGRTCCACCT-CRAARGTC-3′ (positions 21,611 to 21,594). The positions of the PCR primers were numbered according to the complete genome of a HAdV-2 reference strain (GenBank accession number J01917). The HAdV-positive PCR products were purified by gel extraction and then sequenced using a Big-Dye Terminator Ready Reaction Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA) and an automated sequencer ABI 3730 (Applied Biosystems, Foster City, CA, USA). We compared the obtained sequences with the reference sequences of HAdV in GenBank by using the BLAST. The HAdV types could be determined if there was a sequence identity of $\geq$90%.

(4) Phylogenetic study

We constructed a phylogenetic tree based on the nucleotide sequences of the hexon gene of HAdVs. These included 12 different strains (two representative strains of each type) in the study, as well as previous strains isolated in Taiwan and other countries. GenBank accession numbers for the partial nucleotide sequences of the hexon gene generated in this study are MH133244 to MH133255. The phylogenetic tree was built using the neighbor-joining method by the MEGA program 4.0 (Sudhir Kumar, Arizona State University). The reliability of the tree was assessed by 500 bootstrap replicates.

Statistical methods

The Pearson's χ^2 or Fisher exact test was used to compare categorical variables, and the Student's t-test or one-way analysis of variance with Bonferroni-corrected pairwise comparisons for continuous variables. We also carried out multivariate logistic-regression analysis to identify variables that were independently related to the probability of hospitalization for HAdV infection. A p value < 0.05 was considered significant. All statistical analyses were two-tailed and performed with SPSS software, version 25 (IBM Corp., Armonk, NY, USA).

Results

Case number, species and types of adenovirus infection

Overall, a total of 531 cases of HAdV infection were identified during the study period. The numbers and percentages of different HAdV species and types are summarized in Table 1. No instances of co-infection with different HAdV types were noted in the study, as well as repeated HAdV infections. However, there were two HAdV-C isolates indistinguishable between HAdV-2 and HAdV-6, so we sought to differentiate them by the partial nucleotide sequences of the fiber gene.[17] The PCR primers were 5′-TTGTTGCA-GATGAAACGCGC-3′ (positions 31,021 to 31,040) and 5′-GTTTGGAGTCTTGCACGGT-3′ (positions 31,453—31,435). The positions of the PCR primers were numbered according to the complete genome of an HAdV-2 reference strain (GenBank accession number J01917). This method successfully

Table 1 Numbers and percentages of different species and types of human adenovirus identified in this study.

Species and types	Number (% of total)
HAdV-B	387 (73)
HAdV-3	387 (73)
HAdV-C	73 (14)
HAdV-1	20 (4)
HAdV-2	38 (7)
HAdV-5	8 (2)
HAdV-6	7 (1)
HAdV-E	71 (13)
HAdV-4	71 (13)
Total	531

HAdV = human adenovirus.

differentiated the two HAdV-C isolates; both of them were classified as HAdV-2.

Of these HAdV infections, 526 (99.1%) were identified by viral culture alone, 3 (0.6%) were identified by PCR only, and 2 (0.4%) were identified by both culture and PCR. All of the HAdVs were identified from throat swabs. HAdV-E (HAdV-4) were also isolated from nasopharyngeal and rectal swabs in three patients with suspected Kawasaki disease, while conjunctival swabs from these patients gave negative results. Only one patient had confirmed Kawasaki disease and was treated with intravenous immune globulin and aspirin. (Throat, nasopharyngeal, conjunctival and rectal swabs were collected for virus isolation in patients with suspected Kawasaki disease due to a clinical study.) One HAdV-B (HAdV-3) was also isolated from a rectal swab of a patient with acute gastroenteritis and bronchopneumonia; stool bacterial cultures and rotavirus antigen detection were both negative.

Demographic and clinical features

The demographic and clinical features among cases of infection with different HAdV species are shown in Table 2. The age of all patients with HAdV infection ranged from 0.1 years to 18.4 years, with a median age of 4.6 years. The majority of patients (59%) were under five years of age. It was further found that the mean age of patients infected with HAdV-E (HAdV-4) was 1.5 years older than that of patients infected with HAdV-B (HAdV-3) (95% CI, 1.2 to 1.8; $p < 0.001$). This latter group was itself 1.8 years older than that of patients with HAdV-C infection (95% CI, 1.5 to 2.2; $p < 0.001$). Analyzing the data in terms of different HAdV types, however, we found that there was no significant difference between the mean ages of patients infected with different types of HAdV-C.

In terms of clinical manifestations, almost all patients (99%) presented with fever. The average duration of the fever was 4.7 days (range 1—10 days). Most cases (89%) were diagnosed with URI, approximately one fourth of which were complicated by acute otitis media and/or acute sinusitis. Furthermore, 21% of the total patients had LRI. Relatively few patients had a diagnosis of acute gastroenteritis or convulsions/jerks; each only accounted for 5.5% and 1.7% of the total, respectively. In addition, patients with HAdV-B (HAdV-3) infection were associated with a lower incidence of co-infection with other viruses than patients with HAdV-C (HAdV-1, 2, 5) or HAdV-E (HAdV-4) infection ($p < 0.005$ for all pairwise comparisons). However, the pairwise comparison did not reveal a significant difference between HAdV-3 and HAdV-6 infections in the incidence of viral co-infection. The most common co-infecting virus was influenza virus (74%), followed by parainfluenza virus type 3 (8%) and respiratory syncytial virus (8%).

There were 203 (38%) patients developing complications. Patients infected with HAdV-B (HAdV-3) seemed more

Table 2 The demographic and clinical features among patients infected with different adenovirus species.

Variable	HAdV-B[a] (n = 387)	HAdV-C[b] (n = 73)	HAdV-E[c] (n = 71)	p value
Age				
Mean ± SD	4.8 ± 2.5	3.1 ± 2.9	6.9 ± 3.3	<0.001
Median (range)	4.6 (0.1—16.0)	2.4 (0.4—18.4)	6.3 (1.4—15.6)	
Male/Female (ratio)	209/178 (1.2)	44/29 (1.5)	37/34 (1.1)	0.554
Diagnosis, n (%)				
URI	339 (88)	66 (90)	66 (93)	0.374
Complicated URI	87 (23)	9 (12)	16 (23)	0.142
Sinusitis	42 (11)	5 (7)	11 (16)	0.250
Acute otitis media	52 (13)	4 (6)	5 (7)	0.067
LRI	92 (24)	12 (16)	10 (14)	0.100
Bronchitis/Bronchiolitis[d]	28 (7)	5 (7)	4 (6)	0.887
BPN/Pneumonia[e]	64 (17)	7 (10)	6 (9)	0.090
Acute gastroenteritis	23 (6)	2 (3)	4 (6)	0.639
Convulsions/Jerks	6 (2)	3 (4)	0	0.144
Co-infection with, n (%)				
Mycoplasma pneumoniae	3 (1)	1 (1)	0	0.719
Streptococcus pneumoniae[f]	2 (1)	1 (1)	0	0.614
Other viruses	13 (3)	16 (22)	10 (14)	<0.001
Complication, n (%)	159 (41)	20 (27)	24 (34)	0.062
Hospitalization, n (%)	105 (27)	23 (32)	21 (30)	0.713

[a] All HAdV-B were HAdV-3.
[b] HAdV-C included 20 cases of HAdV-1, 38 cases of HAdV-2, 8 cases of HAdV-5, and 7 cases of HAdV-6.
[c] All HAdV-E were HAdV-4.
[d] Including two cases of croup and one case of clinically suspected bacterial tracheitis.
[e] One case of pneumonia was complicated by empyema.
[f] One patient had empyema with positive *S. pneumoniae* antigen tests of urine and pleural fluid; one patient had acute suppurative otitis media with isolation of S. pneumoniae from the ear discharge; and one patient had pneumonia with the detection of *S. pneumoniae* antigen in urine.
BPN = bronchopneumonia; HAdV = human adenovirus; LRI = lower respiratory tract infection; URI = upper respiratory tract infection.

liable to have complications ($p = 0.06$). Sinusitis, acute otitis media and bronchopneumonia were the most common complications, each of which was diagnosed in 11%, 11% and 9% of the total patients, respectively. One particular case among those with complications was a five-year-old boy who presented to the emergency department with fever and abdominal pain. Intussusception was diagnosed and he underwent laparoscopic reduction. HAdV-B (HAdV-3) was later isolated from the throat swab. Because of this, intussusception was regarded in this study as a possible complication of HAdV infection, although we could not ensure a causal relationship. No patients died of HAdV infection during the course of our study.

Comparison between inpatients and outpatients

Patients infected with different HAdV species or types had similar rates of hospitalization ($p = 0.73$ and 0.28, respectively). Table 3 shows the comparison of HAdV infection between hospitalized patients and outpatients. The majority (72%) of patients with HAdV infection were outpatients. Outpatients had a significantly higher probability of being diagnosed with URI, with a total of 97% of outpatients presenting this condition. In contrast, hospitalized patients were more likely to be diagnosed with LRI. Acute gastroenteritis, convulsions, jerks and complications all occurred more frequently in inpatients. Among inpatients, the average duration of their hospital stay was 5.6 days (with a range of 1–84 days). The mean age of inpatients were 1.5 years younger than that of outpatients (95% CI, 1.3 to 1.8; $p < 0.001$).

In a multivariate logistic-regression model, the most crucial risk factors for hospitalization were: underlying neurological abnormalities, prematurity and the diagnosis of pneumonia (Table 4). Seizure disorders represented 92% of underlying neurological diseases, followed by cerebral palsy (31%). Twelve hospitalized patients were born prematurely with an average of 31.4 weeks of gestation (range 26.4–36.3 weeks).

Severe cases requiring intensive care

There were 5 patients (1%) with severe HAdV infection admitted to the intensive care unit (ICU). Table 5 lists the detailed diagnoses, HAdV types and clinical information. The majority (60%) were infected with HAdV-C. Four of the five (80%) were admitted to the ICU due to respiratory failure which required mechanical ventilation, and 1 (20%) was suffered from seizures. All of them had underlying diseases, chiefly neurological and respiratory diseases. Among these, one patient was co-infected with *M. pneumoniae* and *S. pneumoniae*; his disease was complicated by

Table 3 Comparison of clinical features between hospitalized patients and outpatients.

Variable	Outpatients ($n = 382$)	Inpatients ($n = 149$)	p value
Age in year			
Mean ± SD	5.3 ± 2.8	3.8 ± 2.7	<0.001
Median (range)	4.9 (0.5–18.4)	3.3 (0.1–16.0)	
Male/Female (ratio)	205/177 (1.2)	85/64 (1.3)	0.482
Prematurity, *n* (%)	1 (<1)	12 (8)	<0.001
Body weight, *n* (%)			
<3rd percentile	9 (2)	21 (14)	<0.001
>97th percentile	30 (8)	10 (7)	0.654
Underlying diseases, *n* (%)	124 (33)	58 (39)	0.158
Cardiovascular system	11 (3)	9 (6)	0.086
Developmental delay	4 (1)	18 (12)	<0.001
Malignancy	2 (1)	2 (1)	0.314
Neurology system	1 (<1)	12 (8)	<0.001
Allergic rhinitis	65 (17)	15 (10)	0.044
Asthma	63 (17)	19 (13)	0.284
Diagnosis, *n* (%)			
URI	370 (97)	101 (68)	<0.001
Complicated URI	76 (20)	36 (24)	0.279
Sinusitis	42 (11)	16 (11)	0.932
Acute otitis media	40 (11)	21 (14)	0.239
LRI	47 (12)	67 (45)	<0.001
Bronchitis/Bronchiolitis[a]	28 (7)	9 (6)	0.600
BPN/Pneumonia[b]	19 (5)	58 (39)	<0.001
Acute gastroenteritis	14 (4)	15 (10)	0.004
Convulsions/Jerks	3 (1)	6 (4)	0.017
Complication, *n* (%)	112 (29)	91 (61)	<0.001
Secondary bacterial infection, *n* (%)	0	7 (5)	<0.001

[a] Including two cases of croup and one case of clinically suspected bacterial tracheitis.
[b] One case of pneumonia was complicated by empyema.
BPN = bronchopneumonia; LRI = lower respiratory tract infection; URI = upper respiratory tract infection.

Table 4 Risk factors associated with hospitalization for adenovirus infection in a multivariate logistic-regression model.

Variable	Odds ratio	95% CI	*p* value
Age			
4 to <19 year	Reference		
<1 year	7.25	2.82–18.64	<0.001
1 to <2 year	3.46	1.68–7.15	0.001
2 to <3 year	1.09	0.53–2.23	0.821
3 to <4 year	1.53	0.76–3.09	0.233
Body weight			
3rd to 97th percentile	Reference		
<3rd percentile	3.80	1.36–10.64	0.011
>97th percentile	0.76	0.29–1.98	0.573
Prematurity	19.10	2.03–179.38	0.010
Developmental delay	4.56	1.14–18.22	0.032
Malignancy	7.93	1.08–58.29	0.042
Neurological abnormality	24.85	2.72–226.98	0.004
Acute gastroenteritis	4.27	1.79–10.20	0.001
Bronchopneumonia or pneumonia	11.02	5.77–21.07	<0.001

CI: confidence interval.

Table 5 Five cases of severe adenovirus infection.

	Age	Gender	Species (type)	Diagnosis[a]	Indication[b]	Outcome
1	2.79	Male	HAdV-B (HAdV-3)	1. Pneumonia *2. Bronchopulmonary dysplasia due to preterm birth* *3. Infantile spasm*	Respiratory failure	Recovery
2	2.51	Male	HAdV-C (HAdV-2)	1. Lobar pneumonia and empyema, co-infected with *Streptococcus pneumoniae* and *Mycoplasma pneumoniae*, status post VATS decortication *2. Asthma*	Respiratory failure	Recovery
3	9.52	Male	HAdV-C (HAdV-2)	1. Encephalitis with seizure *2. Past history of brain abscess, Streptococcus intermedius, complicated by Broca's aphasia*	Seizure	Recovery
4	2.57	Male	HAdV-E (HAdV-4)	1. Pneumonia, co-infected with *Pseudomonas aeruginosa* *2. Laryngomalacia, status post supraglottoplasty* *3. Developmental delay*	Respiratory failure	Recovery
5	1.04	Male	HAdV-C (HAdV-1)	1. Pneumonia 2. Corneal melting and perforation, right eye, status post corneoscleral patch grafts *3. Multiple congenital anomalies* *4. Infantile spasm* *5. Developmental delay*	Respiratory failure	Recovery

[a] The diagnoses printed in *italics* indicate underlying diseases.
[b] Indication for admission to the intensive care unit.
HAdV = human adenovirus; VATS = video-assisted thoracoscopic surgery.

empyema requiring video-assisted thoracoscopic surgical (VATS) decortication.

Phylogenetic study

We selected the nucleotide sequences of the hexon gene of 12 representative HAdV strains (two representative strains of each type) in the present study, namely, HAdV-1 (GenBank accession numbers MH133244 and MH133245), HAdV-2 (GenBank accession numbers MH133246 and MH133247), HAdV-3 (GenBank accession numbers MH133248 and MH133249), HAdV-4 (GenBank accession numbers MH133250 and MH133251), HAdV-5 (GenBank accession numbers MH133252 and MH133253) and HAdV-6 (GenBank accession numbers MH133254 and MH133255). These were aligned and analyzed with the nucleotide sequences of previous HAdV strains identified in Taiwan, China, Thailand, Korea, Japan and the US between 1953 and 2013.

In the phylogenetic study, we found that the sequences of HAdV-1, HAdV-3, HAdV-4 and HAdV-5 derived from this study clustered with the sequences of the same HAdV types from other studies in Taiwan and other countries, while exhibiting great similarity to those sequences. The phylogenetic tree is shown in Fig. 1.

Discussion

In this study, we reported the molecular epidemiology and clinical features of adenovirus infection in the outbreak of 2014 in Taiwan. Studies have shown that HAdV-3 circulates constantly and can cause outbreaks in communities.[7,18–21] Therefore, it is not surprising that HAdV-3 represented 73% of the total isolates in our study. This finding is also similar to that of the study on the 2011 outbreak in Taiwan, where HAdV3 accounted for 74% of HAdV isolates.[11] However, there is some discrepancy between our results and the data from the nationwide viral surveillance system of the Taiwan Centers for Disease Control.[10] Its data showed that both HAdV-3 and HAdV-4 were the most prevalent types in 2014, each of which accounted for 40% of all HAdV isolates. The rest (20%) were HAdV-2.[10] However, our study only included patients younger than 19 years and from a single medical institution, so this may explain the discrepancy and indicate that there are some regional and age differences in the molecular epidemiology of HAdV infection.

From the analysis of demographic and clinical data, we noted that the mean ages of patients infected with different HAdV species are significantly different. This finding is comparable to other studies, which reported that patients with HAdV-B (HAdV-3) infection are significantly older than those with HAdV-C (HAdV-2) infection.[7,22] In their studies, they did not mention the average age of patients infected with HAdV-E (HAdV-4); however, we found that patients with HAdV-E (HAdV-4) infection are the oldest among the three groups. In addition, we identified that HAdV-B (HAdV-3) infection may be negatively correlated with co-infection with other viruses. However, this relationship still needs further validation since the number of patients with viral co-infections was relatively low.

Based on the analyses in Table 2, clinicians, including those practicing in primary care clinics or a resource-limited setting, can predict the clinical features in cases where the predominant circulating HAdV species is defined. Therefore, we would suggest regular nationwide HAdV surveillance and typing, which is the case in several countries, such as Taiwan,[11,12] Japan[23] and the US.[24] It can not only inform clinical decision making but also provide timely outbreak detection.

When dealing with patients with HAdV infection, special attention should also be given to identify possible complications. This is because, as seen, complications are not uncommon among patients with HAdV infection. For example, 112 patients (21%) had complicated URI and 114 (21%) had LRI (one complicated by empyema). If patients with HAdV infection have persistent fever, otalgia, prolonged purulent nasal discharge, tachypnea or dyspnea, they are more likely to develop complications and therefore warrant appropriate examination and treatment.

We have also identified some crucial risk factors for hospitalization (Table 4). Among inpatients requiring intensive care, underlying systemic diseases play an important role, especially neurological and respiratory abnormalities. Clinical diagnoses of these patients with severe HAdV infection were mainly pneumonia, followed by encephalitis. The findings are comparable to those of the study on the HAdV outbreak of 2011.[9] In the 2011 outbreak, it was also observed that severe cases requiring intensive care were primarily caused by HAdV-7 (49%) and HAdV-3 (42%). The former was seen to lead to more severe infections and a higher mortality rate. In our study, nevertheless, HAdV3 was not seen to cause more severe disease compared with other HAdV types, except for that it seemed more likely to have complications ($p = 0.06$). There was only one severe case caused by HAdV-3. Likewise, Lin CH et al. did not find a higher rate of severe disease in a cluster of HAdV-3 infection.[25] Therefore, the role of HAdV-3 in causing severe disease is not as decisive as HAdV-7. In addition, no HAdV-7 was isolated over our study period, which could explain why there were only five patients (1%) with severe HAdV infection and all of them fully recovered in the present study.

The phylogenetic study showed that the partial sequences of the hexon genes of HAdV-1, HAdV-3, HAdV-4 and HAdV-5 generated from this study exhibit great similarity to the sequences of the same HAdV types from other studies. It may indicate that this part of the hexon genes of these four types (HAdV-1, HAdV-3, HAdV-4 and HAdV-5) is stable and does not show significant variation from that of previous strains from Taiwan or other countries. We also found that two HAdV-C isolates were indistinguishable, namely between HAdV-2 and HAdV-6, by the partial nucleotide sequence of the hexon gene. Weaver et al. made full genome comparisons and discovered that HAdV-2 and HAdV-6 are closely related with 98% homology at the DNA level.[26] Therefore, the sequences of the hexon genes of HAdV-2 and HAdV-6 are sometimes indistinguishable from each other. In this case, other parts of the viral genomes, such as the fiber or penton base genes, should be sequenced to clarify the HAdV type. In this study, we succeeded in using the partial nucleotide sequence of the fiber gene to classify both HAdV-C isolates as HAdV-2. Based on our experience, although HAdV typing by the sequence of partial hexon gene is still widely used,[27,28] we would suggest considering sequencing both hexon and fiber genes wherever the resources are available to improve the accuracy of typing.

The study had a large sample size of patients infected with HAdV and the findings can help clinicians manage HAdV infection better and improve patient outcome. However, there were several limitations to this study. Firstly, as the study was conducted retrospectively, not all the data needed was readily available. Secondly, because the study was carried out in a single institution, it is not clear to what extent the results can be generalized to other regions of Taiwan (or, indeed, other countries). Thirdly, we cannot confirm that all of the diagnoses were caused by HAdV. This is because HAdV may persist as a latent infection and be detected for a long time after an acute infection.[29] Lastly, our abilities to identify some viruses or bacteria are restricted. For example, current routine techniques cannot

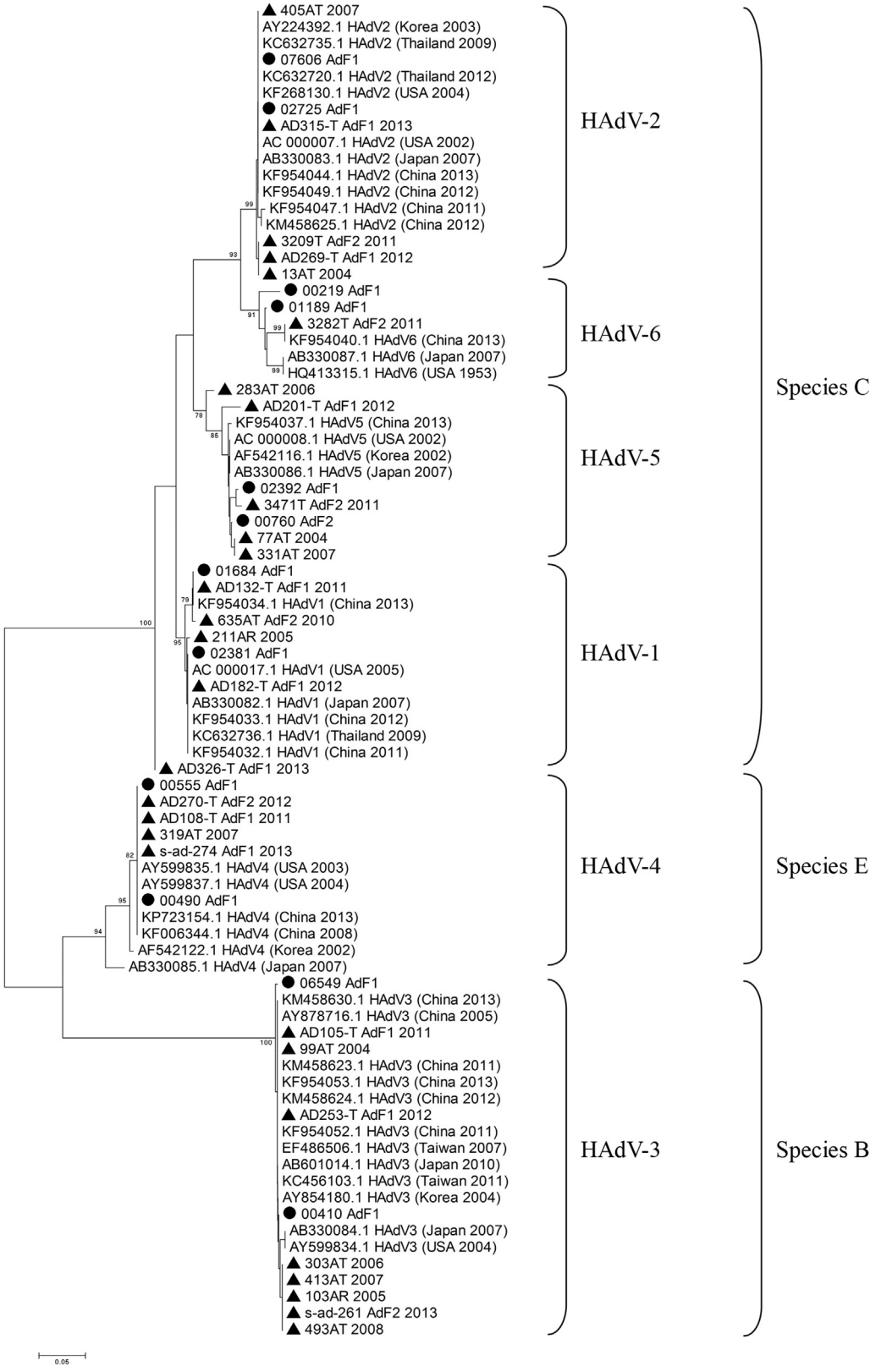

Figure 1. Phylogenetic dendrogram based on the partial nucleotide sequences of the hexon genes of human adenovirus (HAdV) strains. It included different HAdV types from the study (marked with ●), previous strains from Taiwan in different years (marked with ▲), and strains from various countries in different years (country and year indicated in the parenthesis). The numbers at the nodes are percentages indicating the levels of bootstrap support based on a neighbor-joining analysis of 500 replicates.

isolate human bocavirus or human metapneumovirus. While PCRs can be used for this application, they are not routinely performed. In addition, as we have difficulty determining the pathogens causing AOM, acute sinusitis or pneumonia, we can underestimate the incidence of co-infection with other viruses or bacteria.

In conclusion, this study established the molecular epidemiology of the 2014 HAdV outbreak in Taiwan, while demonstrating the correlations between clinical features and different HAdV species.

Conflicts of interest

The authors declare no conflicts of interest.

Author contributions

GLL and LYC conceived and designed the study, performed statistical analyses, interpreted the data, drafted and revised the manuscript. GLL, CYL, JMC, PIL, SYH, KCW, LMH and LYC collected the data and contributed materials. GLL carried out the experiments. All authors read and approved the final version of this manuscript.

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References

1. Lu CY, Huang LM, Fan TY, Cheng AL, Chang LY. Incidence of respiratory viral infections and associated factors among children attending a public kindergarten in Taipei City. *J Formos Med Assoc* 2018;**117**:132–40.
2. Chou CA, Lin TI, Chen YS, Liu PY, Huang YF, Chen YY, et al. Comparisons of etiology and diagnostic tools of lower respiratory tract infections in hospitalized young children in Southern Taiwan in two seasons. *J Microbiol Immunol Infect* 2016;**49**:539–45.
3. Harrach B, Benkö M, Both GW, Brown M, Davison AJ, Echavarría M, et al. Family adenoviridae. In: King AMQ, Lefkowitz EJ, Adams MJ, Carstens EB, editors. *Virus taxonomy: ninth report of the international committee on taxonomy of viruses*. Elsevier; 2012. p. 125–41.
4. Kidd AH, Jönsson M, Garwicz D, Kajon AE, Wermenbol AG, Verweij MW, et al. Rapid subgenus identification of human adenovirus isolates by a general PCR. *J Clin Microbiol* 1996;**34**:622–7.
5. 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. *Eur J Pediatr* 2008;**167**:633–40.
6. 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. *J Med Virol* 2004;**73**:274–9.
7. Wang YF, Shen FC, Wang SL, Kuo PH, Tsai HP, Liu CC, et al. Molecular epidemiology and clinical manifestations of adenovirus respiratory infections in Taiwanese children. *Medicine* 2016;**95**:e3577.
8. 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. *J Med Virol* 2008;**80**:102–12.
9. 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**:e53614.
10. Taiwan Centres for Disease Control. Adenovirus surveillance in Taiwan, 2005–2015. Available at: http://www.cdc.gov.tw/professional/downloadfile.aspx?fid=0AA4EC1D02BB88F8 (accessed on 17th July, 2018).
11. Tsou TP, Tan BF, Chang HY, Chen WC, Huang YP, Lai CY, et al. Community outbreak of adenovirus, Taiwan, 2011. *Emerg Infect Dis* 2012;**18**:1825–32.
12. Taiwan Centres for Disease Control. Top five viruses isolated from respiratory specimens, 2001–2018. Available at: https://data.cdc.gov.tw/dataset/kiho/resource/037336de-ac65-4fad-9c39-2cf5c832ce7a (accessed on 17th July, 2018).
13. Heim A, Ebnet C, Harste G, Pring-Åkerblom P. Rapid and quantitative detection of human adenovirus DNA by real-time PCR. *J Med Virol* 2003;**70**:228–39.
14. Miura-Ochiai R, Shimada Y, Konno T, Yamazaki S, Aoki K, Ohno S, et al. Quantitative detection and rapid identification of human adenoviruses. *J Clin Microbiol* 2007;**45**:958–67.
15. Echavarría M. Adenoviruses in immunocompromised hosts. *Clin Microbiol Rev* 2008;**21**:704–15.
16. Echavarria M, Forman M, Ticehurst J, Dumler JS, Charache P. PCR method for detection of adenovirus in urine of healthy and human immunodeficiency virus-infected individuals. *J Clin Microbiol* 1998;**36**:3323–6.
17. La Rosa G, Della Libera S, Petricca S, Iaconelli M, Donia D, Saccucci P, et al. Genetic diversity of human adenovirus in children with acute gastroenteritis, Albania, 2013-2015. *BioMed Res Int* 2015;**2015**:142912.
18. Cheng JL, Peng CC, Chiu NC, Weng LC, Chiu YY, Chang L, et al. Risk factor analysis and molecular epidemiology of respiratory adenovirus infections among children in northern Taiwan, 2009-2013. *J Microbiol Immunol Infect* 2017;**50**:418–26.
19. Zou L, Zhou J, Li H, Wu J, Mo Y, Chen Q, et al. Human adenovirus infection in children with acute respiratory tract disease in Guangzhou, China. *APMIS* 2012;**120**:683–8.
20. Selvaraju SB, Kovac M, Dickson LM, Kajon AE, Selvarangan R. Molecular epidemiology and clinical presentation of human adenovirus infections in Kansas City children. *J Clin Virol* 2011;**51**:126–31.
21. Qurei L, Seto D, Salah Z, Azzeh M. A molecular epidemiology survey of respiratory adenoviruses circulating in children residing in Southern Palestine. *PLoS One* 2012;**7**, e42732.
22. Lin MR, Yang SL, Gong YN, Kuo CC, Chiu CH, Chen CJ, et al. Clinical and molecular features of adenovirus type 2, 3, and 7 infections in children in an outbreak in Taiwan, 2011. *Clin Microbiol Infect* 2017;**23**:110–6.
23. National Institute of Infectious Diseases. Infectious Agents Surveillance Report. Available at: http://www.niid.go.jp/niid/en/iasr-e.html (accessed on 17 July, 2018).
24. Centers for Disease Control and Prevention. Overview of national adenovirus type reporting system (NATRS). Available at: https://www.cdc.gov/adenovirus/reporting-surveillance/natrs/index.html (accessed on 17th July, 2018).
25. Lin CH, Huang YC, Chiu CH, Huang CG, Tsao KC, Lin TY. A cluster of adenovirus serotype 3 infections in children in northern Taiwan: clinical features and laboratory findings. *J Microbiol Immunol Infect* 2007;**40**:302–9.

26. Weaver EA, Hillestad ML, Khare R, Palmer D, Ng P, Barry MA. Characterization of species C human adenovirus serotype 6 (Ad6). *Virology* 2011;**412**:19—27.
27. Scott MK, Chommanard C, Lu X, Appelgate D, Grenz L, Schneider E, et al. Human adenovirus associated with severe respiratory infection, Oregon, USA, 2013-2014. *Emerg Infect Dis* 2016;**22**:1044—51.
28. Heo JY, Noh JY, Jeong HW, Choe KW, Song JY, Kim WJ, et al. Molecular epidemiology of human adenovirus-associated febrile respiratory illness in soldiers, South Korea. *Emerg Infect Dis* 2018;**24**:1221—7.
29. Fox JP, Hall CE, Cooney MK. The Seattle virus watch. VII. Observations of adenovirus infections. *Am J Epidemiol* 1977;**105**: 362—86.