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Clinical and Molecular Epidemiology of Human Parainfluenza Virus 4 Infections in Hong Kong: Subtype 4B as Common as Subtype 4A▽

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In this 1-year study, 35 (1.2%) of 2,912 nasopharyngeal aspirates were positive for human parainfluenza virus 4 (HPIV4) by reverse transcription-PCR. Patients with HPIV4 infection were mainly young children and immunocompromised adults. In contrast to the reported predominance of HPIV4A infection, molecular subtyping revealed that 15 (44%) cases were caused by HPIV4B.

Although human parainfluenza viruses (HPIVs) are often associated with upper respiratory tract infections, they are also increasingly implicated in more severe disease, especially in immunocompromised patients (5, 15, 19). Based on genotypic and antigenic characteristics, four HPIV serotypes (HPIV1 to -4) are recognized. HPIV4 is further divided into two subtypes, 4A and 4B, by hemagglutination inhibition and neutralizing tests (3).

Owing to the difficulties of cell culture isolation and its absence from the routine panels of respiratory virus antigen detection in most clinical virology laboratories, the incidence of HPIV4 infection is underestimated and its clinical epidemiology is poorly understood (2, 7, 8, 16). We have recently described the first outbreak of HPIV4 infection in Hong Kong, involving 38 institutionalized children and three staff members in November 2004 (10). Reverse transcription (RT)-PCR was also found to be more sensitive (100%) than direct immunofluorescence assay (74%) and cell culture (16%) for diagnosis (10). To date, there has been no RT-PCR-based epidemiology study of HPIV4 infections with clinical data available. Therefore, we carried out a 1-year study on nasopharyngeal aspirates (NPAs) collected from hospitalized patients in Hong Kong.

Patients and microbiological methods. Prospectively collected NPAs from patients admitted to two hospitals, Queen Mary Hospital and Pamela Youde Nethersole Eastern Hospital, in Hong Kong during a 12-month period (April 2004 to March 2005) were included. All NPAs negative for influenza A and B viruses, HPIV1 to -3, respiratory syncytial virus, and adenovirus by direct immunofluorescence assay (20) and human metapneumovirus, human rhinoviruses, and human coronaviruses by RT-PCR (11, 12, 18) were subject to RT-PCR for HPIV4. Once HPIV4 was detected in NPAs, the corresponding patients were identified and the clinical records were retrieved and analyzed.

RT-PCR for HPIV4. NPAs were subject to RNA extraction and RT-PCR for HPIV4 as described previously (1, 10). The nucleotide sequences of the PCR products were compared with known phosphoprotein sequences of members of the family *Paramyxoviridae* by using the ClustalW program. Phylogenetic relationships were determined by using ClustalX version 1.81.

A total of 4,181 NPAs were received during the study period. A total of 2,912 NPAs negative for other respiratory viruses were subjected to RT-PCR for HPIV4. Of the 2,912 NPAs tested, 35 (1.2%) were positive for HPIV4 (Fig. 1). These 35 NPAs were from 34 patients, with 1 patient (case no. 19) having two NPAs positive during the same admission (Table 1). Fifteen (44%) patients had underlying diseases. HPIV4 was found to infect mainly young children (median age, 2 years; range, 1 month to 94 years). Only four patients were adults who had underlying diseases. The three who were over 70 years of age were residents of homes for the elderly, one of whom (case no. 14) reported noticing several other residents with similar fever episodes. HPIV4 was detected throughout the year, except in June and October, with two apparent peaks in summer and late fall, respectively.

The clinical characteristics of patients with NPAs positive for HPIV4 are summarized in Table 1. Of the 34 patients, 16 (47%) had symptoms of upper respiratory tract infection whereas 9 (26%) had pneumonia on presentation. Asthmatic exacerbations or febrile wheeze occurred in eight (24%), four of whom had underlying lung diseases. All three of the adults of advanced age presented with pneumonia. Chest radiography was performed on 26 patients, of whom 15 (58%) showed abnormalities. Five patients (15%) experienced seizures, with one complicated by status epilepticus. All of the patients survived.

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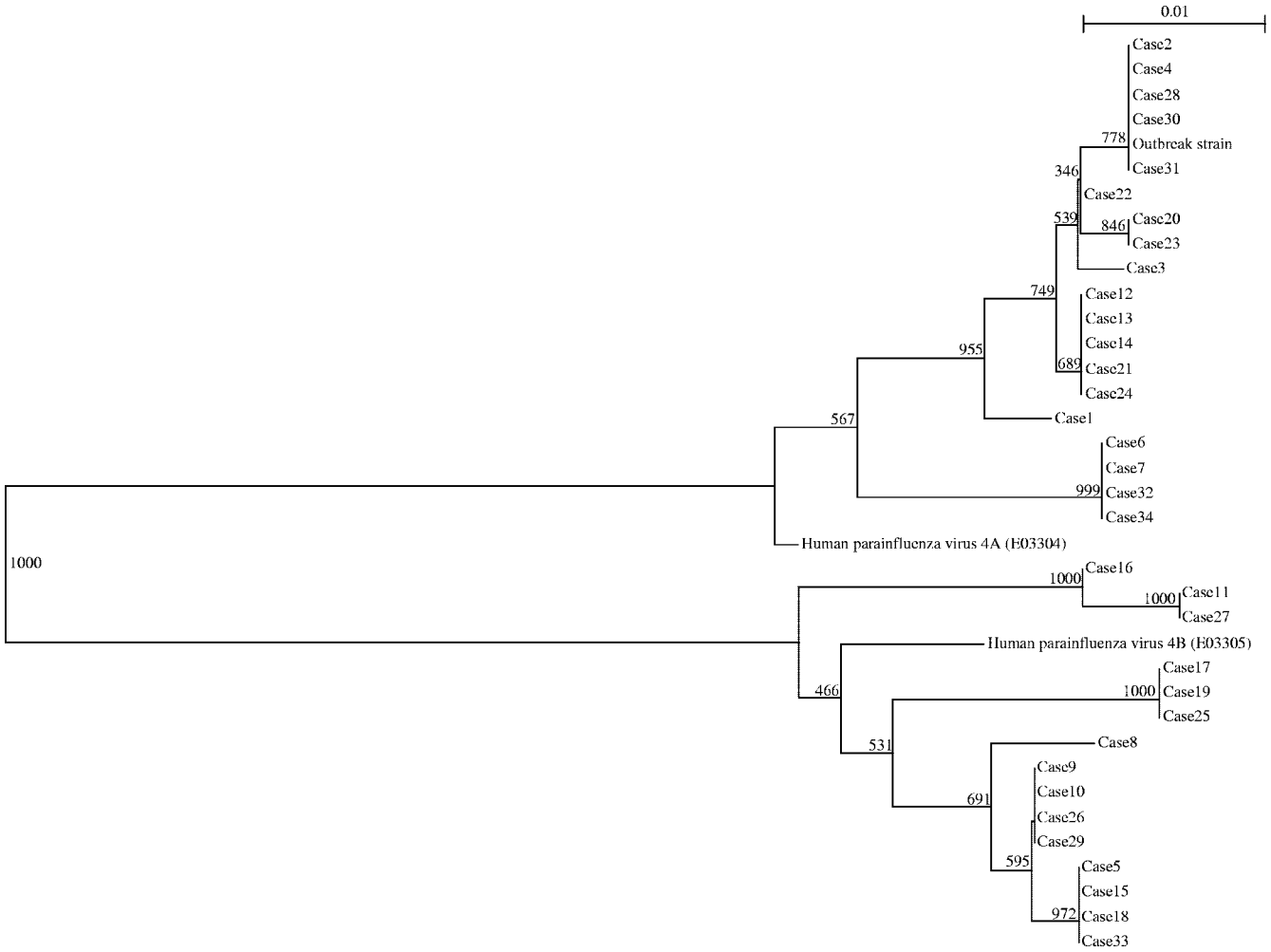

FIG. 1. Phylogenetic analysis of the partial phosphoprotein genes of HPIV4 from the 34 cases studied here. The tree was constructed by the neighbor-joining method by using Kimura's two-parameter correction and bootstrap values calculated from 1,000 trees. A total of 389 nucleotides were included in the analysis. The scale bar indicates the estimated number of substitutions per 100 nucleotides.

Sequencing of the partial phosphoprotein genes showed that 19 (56%) of the patients had HPIV4A infections while 15 (44%) had HPIV4B infections (Fig. 1). No association between genotype and season and severity of illness was observed.

The present study represents the first to describe the epidemiology and clinical manifestations of HPIV4 infections by using RT-PCR as the method for diagnosis. In most epidemiological studies of HPIVs, HPIV3 was the most commonly identified HPIV (>50%), with HPIV4 often representing the most infrequent serotype (1.1 to 10.6%) (2, 6, 13). However, these studies were likely to have been biased, as they were based on viral isolation or rarely direct antigen detection results. In fact, one study has shown that RT-PCR more frequently detected HPIV4 than HPIV2 (1). This is in line with the present study, where 31 cases of HPIV1, 8 cases of HPIV2, and 183 cases of HPIV3 infection were diagnosed by direct immunofluorescence assay during the same period (data not shown). Although we did not perform RT-PCR on HPIV1 to -3 for more accurate comparison, HPIV4 infections may be as common as HPIV1 and -2 infections in our locality.

Description of the clinical manifestations of HPIV4 infection has relied on a small number of reported cases, some with only limited clinical information (2, 4, 6, 13, 14, 16, 17). Here, a significant proportion of the patients, especially those with underlying diseases, had pneumonia and complications such as asthma and seizures. The presentation of pneumonia in three residents of homes for the elderly, all caused by HPIV4 in August, also suggested that HPIV4 may be important in causing pneumonia in the elderly and outbreaks in institutions.

In contrast to the seasonality of HPIV1 to -3, that of HPIV4 is less well described. HPIV4 is often reported during late fall and winter (2, 10, 17). In this study, HPIV4 appeared to be circulated throughout the year. Although apparent peaks were observed in November and July, larger epidemiological studies are required to ascertain its seasonal variations.

HPIV4A was the predominant subtype identified in the few studies where subtyping was performed (1, 9, 17). In a study in Spain, all 10 of the HPIV4-positive samples were subtyped as HPIV4A (1). Among the nine cases of HPIV4 infection reported in Canada, only one strain belonged to HPIV4B (17). Here, 56% of the cases were caused by HPIV4A and 44% were

TABLE 1. Summary of the clinical characteristics of the 33 patients with HPIV4 infections

Case	Mo	Sex[a]/age	Underlying disease(s)[b]	Diagnosis[b]	CXR[d] finding(s)	Genotype
1	April	M/1 yr	None	URTI, encephalopathy	Not done	HPIV4A
2	April	M/3 yr	None	Febrile convulsion	Not done	HPIV4A
3	April	M/5 mo	G6PD deficiency	URTI, febrile wheeze	Clear	HPIV4A
4	May	M/7 mo	None	GE	Clear	HPIV4A
5	May	M/1 yr	None	Febrile wheeze	Perihilar streakiness	HPIV4B
6	July	F/5 yr	None	Febrile illness, epilepsy	Left perihilar streakiness	HPIV4A
7	July	M/2 yr	None	URTI	Left perihilar, LLZ haziness	HPIV4A
8	July	M/5 yr	None	URTI, febrile convulsion	Not done	HPIV4B
9	July	M/6 yr	None	Pneumonia, asthma	Perihilar haziness	HPIV4B
10	July	M/18 yr	Brittle asthma	Asthma	Not done	HPIV4B
11	July	M/11 mo	Ex-prematurity, developmental delay, eczema	Pneumonia	Upper zone haziness	HPIV4B
12	August	F/94 yr	Old CVA, schizophrenia, glaucoma	Pneumonia	Lower zone haziness	HPIV4A
13	August	M/70 yr	Dementia, DM	Pneumonia	RLZ haziness	HPIV4A
14	August	F/72 yr	Dementia, DM, bronchiectasis, cholangitis	Pneumonia	RMZ and RLZ haziness	HPIV4A
15	September	M/2 yr	None	URTI	Clear	HPIV4B
16[c]	November	F/2 yr	Epilepsy	URTI	Clear	HPIV4B
17	November	F/6 yr	None	Pneumonia	Perihilar haziness	HPIV4B
18	November	M/5 mo	None	URTI	Clear	HPIV4B
19	November	M/6 mo	Ex-prematurity, chronic lung disease, IVH	Febrile wheeze	Clear	HPIV4B
20	November	F/7 mo	Neuroblastoma	Tracheitis	Clear	HPIV4A
21	November	M/11 yr	Asthma	Pneumonia, asthma	RUL and RML collapse/consolidation	HPIV4A
22	November	F/5 yr	None	URTI	Clear	HPIV4A
23	November	F/4 yr	None	Pneumonia, gastritis	RLZ haziness	HPIV4A
24[c]	December	F/3 yr	None	Febrile wheeze, rotavirus GE	Not done	HPIV4A
25	December	F/1 mo	None	URTI	Clear	HPIV4B
26	January	M/1 yr	None	URTI, foreign body aspiration	Patchy opacities	HPIV4B
27	January	M/2 yr	Klinefelter syndrome	URTI, UTI	Clear	HPIV4B
28	January	M/6 yr	None	URTI, GE	Not done	HPIV4A
29	January	M/6 mo	None	URTI, rotavirus GE	Not done	HPIV4B
30	Feb	F/4 yr	Febrile convulsion	URTI	Left perihilar shadow	HPIV4A
31	Feb	M/7 yr	None	URTI, drug rash	Peribronchial and left hilar cuffing	HPIV4A
32	March	F/1 yr	Epilepsy	Febrile illness, status epilepticus	Clear	HPIV4A
33	March	F/5 yr	Asthma	Pneumonia, asthma	Perihilar haziness	HPIV4B
34	March	M/4 mo	Choroid plexus cyst, truncal hypotonia	URTI	Not done	HPIV4A

[a] M, male; F, female.
[b] G6PD, glucose-6-phosphate dehydrogenase; CVA, cerebrovascular accident; DM, diabetes mellitus; IVH, intraventricular hemorrhage; URTI, upper respiratory tract infection; GE, gastroenteritis; UTI, urinary tract infection.
[c] Cases with cell culture positive for HPIV4.
[d] CXR, chest X-ray; LLZ, left lower zone; RLZ, right lower zone; RMZ, right middle zone; RUL, right upper lobe; RML, right middle lobe.

caused by HPIV4B, suggesting that both genotypes are important in our population. Further studies are needed to determine their relative importance in other countries.

Nucleotide sequence accession numbers. The nucleotide sequences determined in this study have been lodged within GenBank under accession no. FJ608669 to FJ608702.

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