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Molecular epidemiology of coxsackievirus A6 circulating in Hong Kong reveals common neurological manifestations and emergence of novel recombinant groups

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ABSTRACT

Background: Coxsackievirus A6 (CV-A6) represents the predominant enterovirus serotype in Hong Kong, but its epidemiology in our population was unknown.
Objectives: To examine the clinical and molecular epidemiology of CV-A6 and detect emerging recombinant strains in Hong Kong.
Study design: Nasopharyngeal aspirates (NPAs) from patients with febrile or respiratory illness were subject to RT-PCR for CV-A6 and sequencing of 5′-NCR and VP1. CV-A6-positive samples were further subject to 2C and 3D gene sequencing. Complete genome sequencing was performed on potential recombinant strains.
Results: Thirty-six (0.35%) NPAs were positive for CV-A6 by 5′-NCR RT-PCR and sequencing, 28 of which confirmed by partial VP1 gene sequencing. Among the 28 patients (mainly young children) with CV-A6 infection, hand-foot-and-mouth disease (HFMD) (43%), herpangina (18%) and tonsillitis (11%) were the most common diagnoses. Seven (25%) patients had neurological manifestations, including febrile seizures, encephalitis and meningitis. VP1 gene analysis showed that 24 CV-A6 strains circulating in Hong Kong belonged to genotype D5, while 4 strains belonged to D4. Further 2C and 3D gene analysis revealed eight potential recombinant strains. Genome sequencing of five selected strains confirmed four recombinant strains: HK459455/2013 belonging to recombination group RJ arisen from CV-A6/CV-A4, HK458288/2015 and HK446377/2015 representing novel group RL arisen from CV-A6/CV-A4, and HK462069/2015 representing novel group RM arisen from CV-A6/EV-A71. Recombination breakpoints located at 3D were identified in the latter three recombinant strains, with HK462069/2015 (from a child with encephalitis) having acquired 3D region from EV-A71.
Conclusions: We identified novel recombinant CV-A6 strains in Hong Kong, with 3D being a common recombination site.

1. Background

Human enteroviruses are responsible for wide spectrum of diseases, ranging from febrile exanthema to meningitis and myocarditis. While enterovirus A71 (EV-A71) and coxsackievirus A16 (CV-A16) are the most common pathogens causing hand-foot-and-mouth disease (HFMD) in young children, infections caused by other enteroviruses are increasingly reported. CV-A6, belonging to enterovirus species A (EV-A) is

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a major pathogen of herpangina and an emerging cause of HFMD in Asia [1–4]. CV-A6 infections with neurological involvement, such as acute flaccid paralysis and aseptic meningitis are also increasingly reported [5–8].

Recombination is frequently reported in some enteroviruses such as EV-A71 [9–15]. Recombinant CV-A6 strains have also emerged to cause outbreaks in recent years [1,16–22], and some associated with atypical disease such as eczema coxsackium [23,24]. While CV-A6 represents the most frequently detected EV serotype detected in respiratory samples in Hong Kong (http://www.chp.gov.hk/en/statistics/data/10/641/708/702/5332.html), no viral sequence data were available to assess the molecular epidemiology and possible emergence of recombinant strains [4].

2. Objectives

We examined the clinical and molecular epidemiology of CV-A6 in Hong Kong. CV-A6 strains detected from 28 patients were subject to VP1, 2C and 3D gene sequencing and analysis. Complete genome sequencing and recombination analysis were performed on five selected CV-A6 strains which revealed novel recombinant viruses.

3. Study design

3.1. Patients and microbiological methods

Nasopharyngeal aspirates (NPAs) were collected from patients of two regional hospitals from January 2010 to December 2017 [25]. The study was approved by the Institutional Review Board of the University of Hong Kong/Hospital Authority Hong Kong West Cluster (no. UW 16-365) and Research Ethics Committee of Hong Kong East Cluster (no. HKEC-2016-041).

3.2. RT-PCR for detection of CV-A6

RNA extraction from NPAs, RT-PCR and sequencing for EVs were performed using primers targeted to the 5′-non-coding region (5′-NCR) (Table S1, Supplementary Data) [26].

3.3. RT-PCR and sequencing of partial VP1, 2C and 3D genes and phylogenetic analysis

Positive samples with partial 5′-NCR sequences potentially belonging to CV-A6 were subject to RT-PCR and sequencing of partial viral protein 1 (VP1) gene for species confirmation (Table S1, Supplementary Data) [26]. Samples containing CV-A6 as confirmed by VP1 gene sequencing were further subject to RT-PCR and sequencing of partial 2C and 3D genes. Phylogenetic trees were performed by neighbour-joining method using Jukes-Cantor method in MEGA 7.0 [27].

3.4. Selective pressure analysis of VP1 genes

The ratio of non-synonymous to synonymous substitution ($\omega = dN/dS$) was estimated to measure the individual site-specific selection pressure of CV-A6 VP1 and implemented in Hypothesis testing using Phylogenies (HyPHy) package [26,28,29].

3.5. Complete genome sequencing of potential recombinant CV-A6 strains

The genomes of five selected CV-A6 strains, including four potential recombinant strains, were amplified and sequenced [14,15] (Supplementary Table S1). 5′/3′ ends were confirmed by rapid amplification of cDNA ends using 5′/3′ RACE kit (Roche, Germany). Sequences were edited by SeqMan software of DNASTAR Lasergene package to achieve final genome sequences.

3.6. Genome and recombination analysis

Genome sequences were compared to available enterovirus sequences in GenBank (Table 2, Supplementary Data). Recombination analysis was conducted using nucleotide alignment generated by MEGA7 [30]. Genetic algorithms for recombination detection (GARD) method was used to detect putative breakpoints in Datamonkey [31]. Similarity plot and bootscanning analyses were performed using SimPlot software version 3.5.1 [32]. Phylogenetic analysis around recombination sites was performed. The best evolutionary model was determined using jModelTest 2.1.10 [33].

3.7. Nucleotide sequence accession number

The 5′-NCR, VP1, 2C, 3D and genome sequences were deposited in GenBank (MH049744–MH049868) (Table 3, Supplementary Data).

4. Results

4.1. Detection of CV-A6 from nasopharyngeal aspirates and clinical characteristics of patients with CV-A6 infection

10,400 NPAs from patients hospitalized for febrile or respiratory illness from January 2010 − December 2017 were subject to RT-PCR and sequencing of partial 5′-NCR for detection of EVs. Thirty-six (0.35%) NPAs were positive for CV-A6 by 5′-NCR sequencing (Fig. S1, Supplementary Data), and subject to RT-PCR and sequencing of partial VP1 gene for species confirmation. NPAs potentially containing other EVs were being subject to investigations and not included here. Partial VP1 gene was successfully amplified and sequenced in 28 samples, with ≥82.9% nucleotide identities to known CV-A6 sequences. The annual incidence of CV-A6 among tested samples was 0.33% (5/1500), 0.27% (4/1500), 0.15% (2/1300), 0.31% (4/1300), 0.08% (1/1200), 0.83% (10/1200), 0.50% (6/1200), and 0.33% (4/1200) from 2010 to 2017 respectively, with peaks during mid-summer and mid-autumn (June and October) (Fig. 1).

The clinical characteristics of the 28 patients with CV-A6 infection are summarized in Table 1. Except for a 25-year-old woman (patient 20), all patients were young children (23-day- to 6-year-old) with male-to-female ratio of 22:6. Four patients had recent contact with HFMD in the household (patients 3, 12, and 26) or community (patient 11). Household contacts of six other patients (patients 2, 5, 8, 14, 16 and 20) also had recent febrile or upper respiratory tract illnesses. One patient (patient 9) resided in Shenzhen, China. Two other patients (patients 5 and 12) had recent travel to mainland China.

Sixteen patients had underlying diseases. Twelve patients presented with HFMD (43%), five with herpangina (18%) and three with tonsillitis (11%) as the most common diagnoses. Non-specific viral exanthema was diagnosed in four patients (14%). Features of neurological involvement were present in seven patients (25%), including four patients with febrile seizures (14%), one with encephalitis and two with

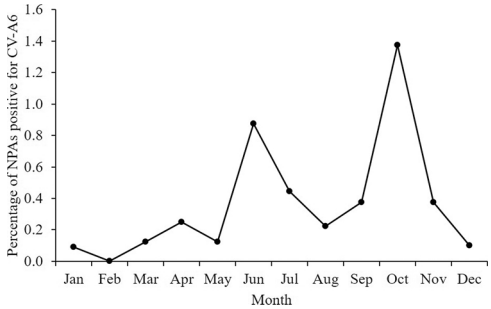

Fig. 1. Monthly distribution of CV-A6 in Hong Kong from 2010 to 2017. The line depicts the percentage of positive NPAs each month.