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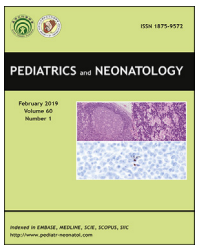

Short Communication

Outbreak of recombinant coxsackievirus A2 infection and polio-like paralysis of children, Taiwan, 2014

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1. Introduction

Patients with coxsackievirus A2 (CVA2) infection mostly present with herpangina and rarely have central nervous system complications.[1] However, in 2012, a Hong Kong study reported that a naturally occurring recombinant CVA2 caused an outbreak of severe respiratory symptoms, leading to two deaths.[2] In the current study, we report an outbreak of CVA2-infection-related acute flaccid paralysis (AFP) in Taiwan. Sequencing and analysis of the viruses isolated from fecal samples revealed that the currently prevalent CVA2 strain is highly similar to the Hong Kong recombinant.

2. Methods

Between April and August 2014, enterovirus infection outbreaks were reported in Taiwan. According to the disease surveillance system for enterovirus (EV) infections of the Taiwan Centers for Disease Control (CDC) and Taiwan National Infectious Disease Statistics System, enteroviruses were the cause of 191 808 emergency consultations, 365 131 outpatient department visits, and 5881 hospitalizations. The outbreak peaked in mid-June 2014 (week 23), in which 524 herpangina-related hospitalizations were reported. Younger patients in central Taiwan had higher hospitalization rates than those in other areas of Taiwan. CVA2 was most commonly isolated from patients at that time (Figure S1)[1].

According to the National AFP Surveillance System records, four confirmed polio-like asymmetric paralysis cases were reported during the outbreak. All four cases were

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[1] Taiwan Centers for Disease Control and Prevention. Available at http://www.cdc.gov.tw/diseasesurveillance/enterovirus.

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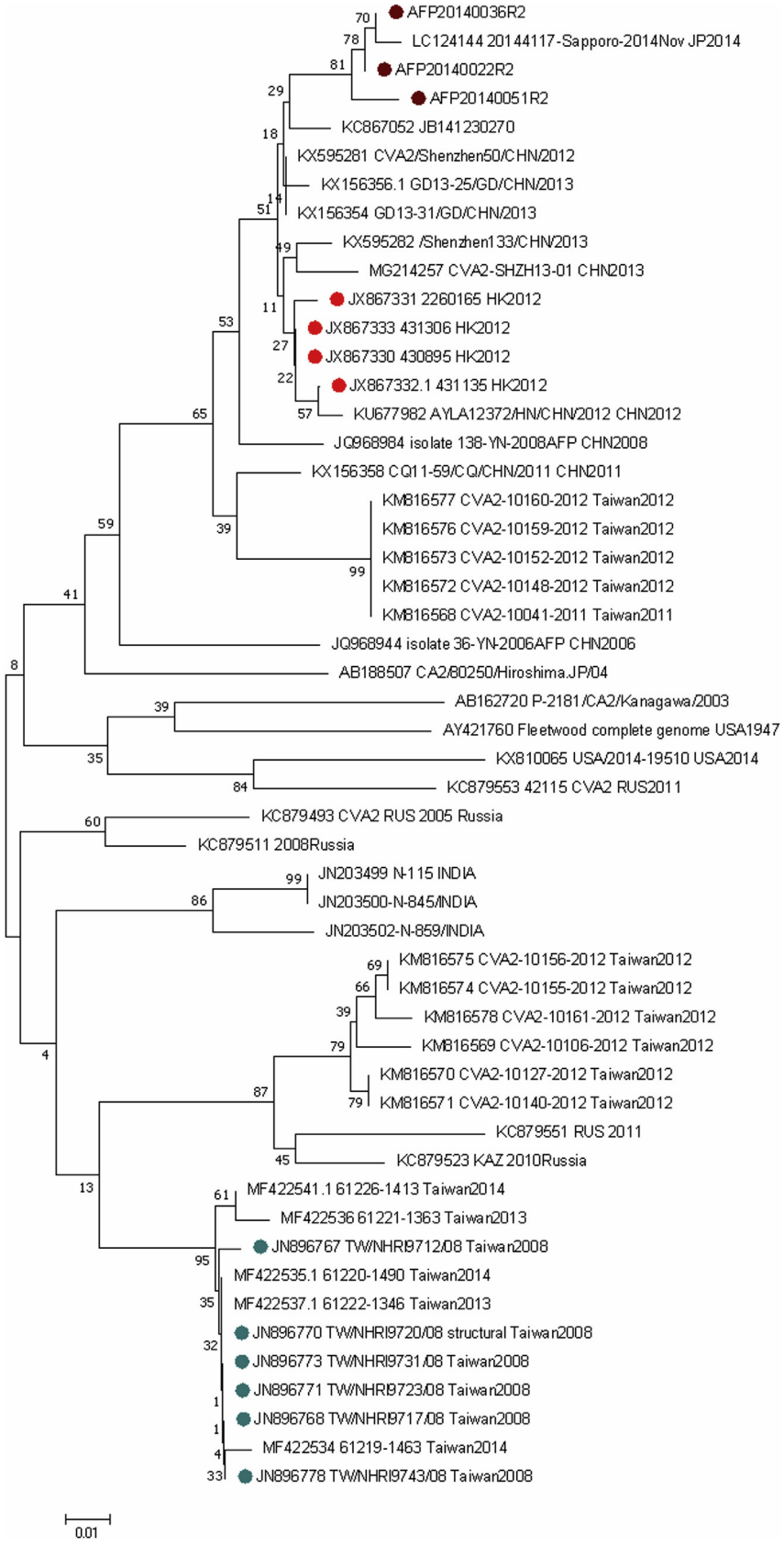

Figure 1 Evolutionary relationships of taxa from different geographical regions, based on the partial VP1 gene sequence. Green dots: strains isolated in Taiwan in 2008; red dots: HK2012 strains; and brown polka dots: 2014 Taiwan AFP strains. The evolutionary history was inferred using the neighbor joining method. The bootstrap consensus tree was inferred from 1000 replicates. Branches corresponding to partitions reproduced in <70% of bootstrap replicates were collapsed. Evolutionary analyses were conducted using MEGA7.

observed in young children living in central Taiwan, and three of the four cases were revealed to be CVA2 infections. The identity of CVA2 was confirmed using PanEV RT-PCR after viral isolation. These children had all recovered from a nonspecific viral infection episode within 1 week prior to their admission. AFP was observed in the upper extremity of one boy, unconsciousness and weakness in the left limbs of a 9-month-old baby girl, and AFP in the lower extremities of the two other boys. The two boys were aged 25 months and 4 years old. Both the boys initially received diagnoses of herpangina, but they subsequently exhibited unilateral lower limb weakness. However, the stool isolation results of the 4-year-old boy were negative for CVA2.

The most remarkable case was that of a 23-month-old boy with AFP of the left upper extremity. His medical history revealed that he was admitted 6 days previously for febrile acute bronchiolitis with respiratory distress. Herpangina was not observed, and he recovered satisfactorily 2 days after admission. On readmission, tenderness was observed over his left forearm and wrist. A neurological examination revealed that he was unable to lift his left arm. The decreased muscle tone of his left arm was evident, and no movement was observed in his left fingers. Deep tendon reflexes were absent in the left arm; however, the other sensations appeared intact. A cerebrospinal fluid examination revealed 13 cells/mm^3 of white blood cells with 98% mononuclear cells. Nerve conduction studies revealed complete conduction block, and hyperintense lesions of the bilateral anterior horn cells were observed on T2-weighted magnetic resonance images of the cervical spinal cord. His neurological condition had deteriorated by the third day of admission, and he exhibited a wide-based unsteady gait. Pulse therapy with methylprednisolone was administered. He was discharged 8 days after admission. The ataxia of both his lower limbs had almost completely recovered; however, minimal neurological improvement was observed in his left upper limb. At the 6-month clinical follow-up, three patients exhibited residual motor weakness.

To understand the molecular basis for the possible pathogenic mechanisms of the CVA2 strains, viral RNA was extracted from the virus cultures isolated from the stool samples. The viral protein 1 (VP1) gene was amplified using a reverse-transcriptase polymerase chain reaction. We constructed a CV2 phylogenetic tree of Taiwan based on the partial VP1 region, using the maximum likelihood method implemented in the DNAml program of PHYLIP 3.6. The topology of the trees was obtained and bootstrapped for 1000 replicates. A bootstrap value greater than 80% was considered to indicate that the sequences belonged to the same group or cluster as a homologous source.

3. Results and discussion

The tree revealed that the AFP strains were close to other CVA2 lineages recently circulating in Taiwan, particularly to those of 2013 and 2014, but they were distinct from the 2008—2010 lineages (Figure S2). The VP1 sequences of the three CVA2 strains exhibited a nucleotide (nt) sequence identity match of only 83%—87% with the 2008—2010 CVA2 lineages.

We compared the VP1 sequences of the patients with AFP with the VP1 sequences of all CVA2 serotypes in the public gene database by using the BLAST and nucleotide BLAST tools of the National Center for Biotechnology Information.[2] Only 80%—87% of the nts of the resulting VP1 sequences were identical to those of the major worldwide CVA2 strains documented before 2012 in the GenBank database. Compared with a 2008 CVA2 strain in Taiwan (TW/NHR NHRI9720/08), the sequence identity was 86%—87% at the nt position 7—407. However, when compared with the two Taiwan EV71 strains (TW-2008-03352 and TW-2006-01741), the sequence identity match was 85%—87%.

We established another phylogenetic tree by using numerous CVA2 strains occurring in different geographical regions of the world to understand the evolutionary relationships between the strains. The results revealed that two linages of CVA2 have been circulating in Taiwan since 2011 (Fig. 1). The 2014 AFP strains belong to the same lineage as the Hong Kong strain identified in 2012 (HK2012 strain), which caused two deaths. These AFP strains also shared a common lineage with strains from South China and Sapporo. The sequence identity match of the VP1 gene of the 2014 AFP strains was 96%—97% with that of the HK2012 strain.

The possibilities of recombination events involving the current CVA2 strain were evaluated using complete alignments and similarity plots. The complete genome of 2014 AFP CVA2 strain was compared with the complete genomes of the CVA2, CVA10, CVA4, and EV71 prototypes; some earlier and recent local (from Taiwan) EV and CV strains, including the HK2012 CVA2 strain that caused an outbreak; and some 2013 and 2014 CVA2 strains from South China. Our results showed that the Taiwan 2014 AFP strain is distinct from the CVA2 prototype (CVA2-AY421760-Fleetwood strain), and from the earlier Taiwan CVA2 strains (2002, 2007, and 2008) and some current strains in Taiwan (2013 and 2014) (Fig. 2). However, we found the 2014 AFP strain exhibits 98% similarity with the HK2012 strain. Furthermore, the sequence identity match was >96% even in the P3 region. This HK2012 strain has been proved to be a natural recombinant. It was characterized using complete genome sequencing and was found to be a recombinant virus of at least 3 EVs (CVA2, EV71, and CVA4) that exhibited the CVA2 capsid.[2] However, our current data did not support the hypothesis that this strain underwent recombination with other EV strains in Taiwan. The closest estimate is that the recombination occurred previously but was detected in Hong Kong in 2012. The HK2012 recombinant CVA2 was somehow introduced into Taiwan, where it caused an outbreak. In addition, based on the results of our evolutionary analysis and nt similarity analysis, we believe that two CVA2 genotypes have been circulating in Taiwan since 2011—2012.

Studies have demonstrated that several human EV A viruses of the same or different serotypes cocirculate during outbreaks, and mixed infections with two or three serotypes in the same patient are common.[4,5] In 2011, a complete genome analysis of the cocirculating EVs reported that the EVs had undergone a recombination event that produced new virus variants.[6] We strongly suspect that the recombinant CVA2 isolated in Taiwan was generated under such conditions. In addition, we found that two genotypes of CVA2 are circulating in Taiwan (Fig. 2), and a study in China reported a similar situation.[7]

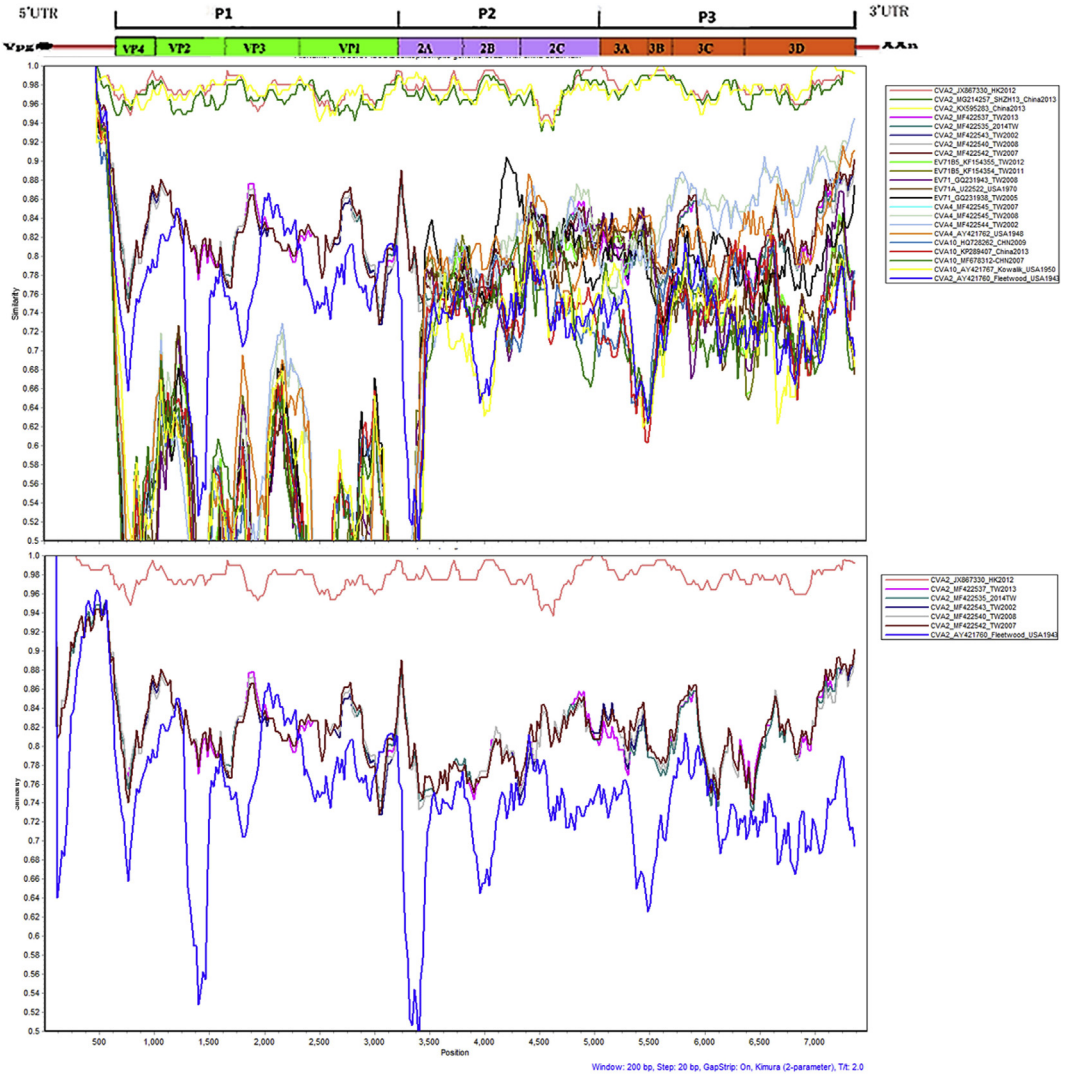

Figure 2 Nucleotide similarity plot of complete genome of CVA2 with the current strain as the query sequence. The 2014 AFP strain exhibited 98% similarity with the HK2012 strain, and the sequence identity match even at the P3 position was higher than 96%. Two genotypes of CVA2 are circulating in Taiwan. (Simplot version 3.51, Kimura mode, Ts/Tv ratio: 2.0).

In conclusion, we report a recombinant CVA2 isolated from children with AFP in Taiwan. Because the CVA2 has an outbreak potential, and this CVA2 strain has a high risk of unfavorable neurological outcomes, children with AFP should be examined for the presence of CVA2 and other pathogenic EVs. Monitoring fatal-case numbers of EV infections without genotyping analysis cannot cease EVs rapidly evolving and widely spreading.

Conflicts of interest

The authors declare no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.pedneo.2018.02.003.