Complete Genome Sequence of a Human Enterovirus 71 Strain Isolated from a Fatal Case in Shanghai, China, in 2012

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The complete genome sequence of a human enterovirus 71 strain (SH12-276), isolated from a fatal case in Shanghai in 2012, was determined. Phylogenetic analysis based on the complete genome sequence classified this strain into subgenotype C4.

H

uman enterovirus 71 (EV71), a member of the human en-

terovirus A species of the family Picornaviridae, is one of the

main causative agents of hand, foot, and mouth disease (HFMD)

in children and infants, predominantly in the Asia-Pacific region

(1–4). In particular, EV71 has been associated with more severe

cases such as aseptic meningitis, encephalitis, or even death (5–7).

Children become susceptible to severe EV71 infections after the

loss of maternal antibodies, and one- to two-year-old children are

most at risk (8, 9). The genome of EV71 is about 7.4 kb, consisting

of a 5’-untranslated region (5’-UTR), P1, P2, P3, and a 3’-

untranslated region (3’-UTR) (10). EV71 can be phylogenetically

classified into 3 main genogroups (A, B, and C) and 11 genotypes

(A, B1 to B5, and C1 to C5) (11). Understanding of the genotypes

of EV71 strains in the epidemic regions is important for the devel-

opment of novel strategies for the prevention and treatment of the

diseases associated with EV71.

In this study, a rectal swab was collected from a 6-month-old

infant with a clinical diagnosis of hand, foot, and mouth disease at

the Children’s Hospital of Fudan University. She had fever, sparse

rash on the feet and buttocks, and an oral ulcer for 2 days before

admission to the hospital. She developed recurrent vomiting,

tachycardia, tachypnea, hypoxemia, and hyperglycemia, and died

3 h after hospitalization. The pathogen was identified as EV71 by

means of real-time reverse transcription (RT)-PCR. The clinical

isolate was obtained by culturing the clinical sample in RD cells for

up to 5 passages, followed by plaque purification. A total of nine

sets of primers were designed to amplify the full genome by reverse

transcription-PCR, which was available upon request. Sequence of

the 5’-end was determined by using the 5’/3’ rapid amplification

denaturing (RACE) kit, 2nd generation (Roche), according to the

manufacturer’s instructions. The gel-purified RT-PCR products were

subject to Sanger sequencing using an ABI 3730xl automatic DNA

analyser. The whole genome of this virus was established by assembling overlapping fragments using the Seq-

Man program of the Lasergene 7 package (DNASTAR).

The full-length genome of the EV71 strain SH12-276 was com-

posed of 7,405 nucleotides (nt), excluding the poly(A) tail. The

5’-UTR was found to be 742 nt, followed by an open reading

frame (ORF) encoding the structural protein P1 (2,586 nt), the

nonstructural proteins P2 (1,734 nt) and P3 (2,259 nt), and the

3’-UTR (81 nt). The contents of A, G, C, and U were 27.12%,

23.86%, 23.93%, and 25.09%, respectively, with G+C contents of

47.79%. Phylogenetic trees were constructed by means of the

neighbor-joining method with the use of MEGA software, version

5.0, to estimate the viral gene relationship with selected enterovi-

rus strains obtained from GenBank. The results of phylogenetic

analyses suggest that SH12-276 belongs to subgenotype C4. Fur-

thermore, SH12-276 was found to be closely related to strain 35/

Jingdezhen/China/HFMD_Severe/2011 (GenBank accession no.

JQ806378 [98.2% nucleotide identity]) and strain SD09-14/SD/

CHN/2009 (GenBank accession no. JX678883 [98.1% nucleotide

identity]), which were isolated from different geographic regions in

China.

Nucleotide sequence accession number. The full-length se-

quence of SH12-276 isolated in Shanghai in 2012 was deposited in

GenBank under the accession no. KC570453.

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