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GENOME ANNOUNCEMENT

Complete Genome Sequence of a Recombinant Coxsackievirus B4 from a Patient with a Fatal Case of Hand, Foot, and Mouth Disease in Guangxi, China

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The coxsackievirus B4 (CVB4) belongs to human enterovirus B species within the family *Picornaviridae*. Here we report a novel complete genome sequence of a recombinant CVB4 strain, CVB4/GX/10, which was isolated from a patient with a fatal case of hand, foot, and mouth disease in China. The complete genome consists of 7,293 nucleotides, excluding the 3′ poly(A) tail, and has an open reading frame that maps between nucleotide positions 742 and 7293 and encodes a 2,183-amino-acid polyprotein. Phylogenetic analysis based on different genome regions reveals that CVB4/GX/10 is closest to a CVB4 strain, EPIHFMD-CLOSE CONTACT-16, in the 5′ half (VP4∼2B) of the genome, although it is closer to a Chinese CVB5 strain, CVB5/Henan/2010, in the 3′ half (2C∼3D) of the genome. Furthermore, similar bootscan analysis based on the whole genomes demonstrates that recombination has possibly occurred within the 2C domain and that CVB4/GX/10 is a possible progeny of intertypic recombination of the CVB4 strain EPIHFMD-CLOSE CONTACT-16 and CVB5/Henan/2010 that occurred during their cocirculation and evolution, which is a relatively common phenomenon in enteroviruses.

Coxsackievirus B4 (CVB4) belongs to the genus *Enterovirus*, family *Picornaviridae*, and is one of six serotypes of the coxsackievirus B group (11). CVB4 can cause a broad range of diseases, such as myocarditis, pancreatitis, hepatitis, aseptic meningitis, meningoencephalitis, gastroenteritis, necrotizing enterocolitis, pneumonia, and even death in neonates. Also, CVB4 has been reported to be implicated as an etiological agent for type 1 insulin-dependent diabetes mellitus (IDDM) (1, 2, 4, 8, 11).

In April 2010, a child with hand, foot, and mouth disease complicated with aseptic meningitis and myocarditis was admitted to hospital in Guangxi, China, and died a few days later. Enterovirus in throat swabs and serum was detected by reverse transcription-PCR (RT-PCR) (9). The virus was identified as coxsackievirus B4 through sequencing of the VP1 gene, isolated using the RD cell line from throat swabs, and named CVB4/GX/10.

Here, we report the complete genome of CVB4/GX/10 as sequenced using overlapping primers. Genome ends were acquired using a rapid amplification of cDNA ends (RACE) kit (Invitrogen). PCR products of the expected sizes were sequenced with an Applied Biosystems 3730 Sanger-based DNA analyzer, and contigs with high-quality trace files were assembled using vNTI (Invitrogen).

The complete genome of this virus consists of 7,293 nucleotides (nt), excluding the 3′ poly(A) tail. Analysis of the sequence demonstrated the presence of a 741-nt 5′ untranslated region (UTR), a 91-nt 3′ UTR, and an open reading frame (ORF) that maps between positions 742 and 7293 and encodes a 2,183-amino-acid polyprotein. The genome organization of this virus is identical to that of previously published CVB4 strains (2).

The P1 (VP4, VP2, VP3, and VP1), 2A, and 2B regions of the genome all show the highest nucleotide acid sequence identity (98%) to those of a CVB4 strain, EPIHFMD-CLOSE CONTACT-16 (GenBank accession no. JN016524.1), which confirms the serotype of the virus as CVB4, although CVB4/GX/10 is closest to a Chinese CVB5 strain, COXB5/Henan/2010 (GenBank accession no. HQ998851), with 93% and 98% nucleotide acid sequence homology in the 2C and P3 (3A, 3B, 3C, and 3D) regions, respectively. Phylogenetic analysis based on different genome regions, such as the VP1 gene and 3D gene, respectively, was performed (10), and the results indicated that although CVB4/GX/10 was closest to EPIHFMD-CLOSE CONTACT-16 CVB4 in the 5′ half (VP4∼2B) of the genome, it was closer to COXB5/Henan/2010 (GenBank accession no. HQ998851) in the 3′ half (2C∼3D). Furthermore, a similar bootscan analysis (6) based on available genomes, such as the genomes of CVB4/GX/10 in this study, CVB4/J.V.B. Benschoten (GenBank accession no. X05690), CVB4/Tuscany (GenBank accession no. DQ480420), the CVB4/E2 variant (GenBank accession no. AF311939), and CVB5/Henan/2010 (GenBank accession no. HQ998851.1), demonstrated that recombination has possibly occurred within the 2C domain and that CVB4/GX/10 is a possible progeny of intertypic recombination of the CVB4 strain EPIHFMD-CLOSE CONTACT-16 and CVB5/Henan/2010 during their cocirculation and evolution, which is a relatively common phenomenon in enteroviruses (3, 5, 7, 12, 13).

Nucleotide sequence accession number. The genome sequence of CVB4/GX/10 has been deposited in NCBI GenBank under accession no. JX308222.

ACKNOWLEDGMENT

This work was supported by the National Basic Research Program (grant no. 2011CB504902) from the Ministry of Science and Technology of China, National Science and Technology Key Projects on Major Infec-

Received 16 July 2012 Accepted 20 July 2012

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doi:10.1128/JVI.01808-12

tious Diseases such as HIV/AIDS, Viral Hepatitis Prevention and Treatment (2011ZX10004-001).

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