Identification of the First C1 Subgenotype of Enterovirus 71 in Mainland of China in a Retrospective Study

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Abstract

The C4 subgenotype of EV71 is identified as the most dominant subgenotype circulating in Chinese mainland since 1998, while the circulation situation of EV71 before 1998 is not well established due to insufficient experimental data. The C1 subgenotype of EV71 has not been reported in Chinese mainland by now. Based on AFP surveillance system of the mainland of China, this study performed a retrospective study of AFP cases for 1985-1999，A strain of EV-A71 C1 subgenotype was found. To our knowledge, this strain (SD92-41) is the first C1 subgenotype reported in Chinese mainland. This study proves that the C1 gene subtype appeared in Chinese mainland, but it is not clear whether it is an imported or a local epidemic strain. With sufficient information from retrospective studies, the source of the SD92-41 strain will be identified, and the prevalence of EV-A71 in Chinese mainland before 1998 will be clearer.

Introduction

As a member of the human Enterovirus species A (genus *Enterovirus*, family *Picornaviridae*), Enterovirus 71 (EV71) is a small, non-enveloped, positive-stranded RNA virus. Based on the VP1 coding region, the worldwide circulation of EV71 can be divided into seven genotypes designated A to G, where the two major genotypes B and C are classified into 14 subgenotypes respectively designated B0 to B7 and C1 to C6[1]. The subgenotype C4 has been identified as the most dominant subgenotype circulating in Chinese mainland since 1998, however the circulation situation of EV71 before 1998 is not well established due to insufficient experimental data[2]. It is reported that the subgenotype C1 of EV71 circulated only in the Western Pacific, Europe and the United States before 2000. After 2000, it was reported that C1 genotype was prevalent in Southeast Asia, such as Malaysia, Thailand, Hong Kong, however the subgenotype C1 of EV71 has not been reported in Chinese mainland by now[3]. Based on AFP surveillance system of the mainland of China，this study performed a retrospective study of AFP cases for 1985-1999，A strain of EV-A71 C1 sub-genotype was found.

Material And Methods

172 human rhabdomyosarcoma (RD) cell positive virus isolates from AFP surveillance system in Chinese mainland from 1985 to 1999 were retrospectively studied and molecular typing method was performed. Primer pair Y7/Q8 was used for poliovirus screening, and for the isolates with negative results, primer pairs 486/488 and 040/011 were used to amplify the partial VP1 sequences and the combination of the two sequences yielded the entire VP1 coding region. 164 polioviruses and 8 (4.7%) NPEV strains were identified. 8 NPEVs included 2 Coxsackievirus B5, 2 Echovirus 1, 1 EV71, 1 Echovirus 26, 1 Coxsackievirus B1 and 1 Enterovirus-B.

Results

The EV71 strain (named SD92-41) was isolated from a 7-year-old male patient with AFP in Shandong Province in 1992. The AFP case was diagnosed as Guillain-Barre syndrome (GBS) by a polio diagnosis

expert panel, and the patient had no residual paralysis during the 60-day clinical follow-up. Phylogenetic analysis based on entire VP1 coding regions of EV71 was conducted with the maximum-likelihood (ML) method revealed that the SD92-41 strain belonged to the C1 subgenotype（Fig.1）. The VP1 genome sequence of the SD92-41 strain and the EV-71 prototype strain showed 80.8% similarity in nucleotide sequence and 96.9% similarity in amino acid sequence. Homologous comparison revealed that the SD92-41 strain had the highest homology with the strain 9718-TX-89 from U.S. in 1989 and had 96.2% similarity in nucleotide sequence and 99.7% similarity in amino acid sequence.

The whole genome sequence of the SD92-41 strain was determined to be 7411 nt long. The ORF of the SD92-41 strain is 6582 nt in length, encoding a polypeptide of 2193 amino acids, with a 746nt 5’-UTR and a 83nt 3’-UTR. Phylogenetic trees based on VP1, P1, P2, P3 coding regions of prototypes strains of the human enterovirus species A and EV71 typical strains were constructed with the maximum-likelihood (ML) method (Fig 2). The topology of the phylogenetic tree based on the nucleotide sequences of the P1 region was similar to that of the VP1 region ,the SD92-41 strain and other subgenotypes of EV71 were clustered into a large cluster (fig 2a and fig 2b）. However, SD92-41 and CVA8 were clustered into one branch in P2 and P3 regions, indicating that SD92-41 and CVA8 may be recombined. Simplot analysis showed that SD92-41 and CVA8 had highly gene recombination in P2 and P3 regions (Fig.3).

Discussion

Since 1998, the C4 subgenotype strains of Enterovirus 71 have been persistently circulating in Chinese mainland for 22 Years [2], where only one or two imported subgenotypes were reported [4]. Reports on the circulation situation of EV71 before 1998 are few. One sequence from Heilongjiang in 1997 and one sequence from ShanDong in 1996 were identified as C3 and C2 subgenotype respectively, indicating that the C3 and C2 subgenotypes appeared in mainland of China [5,6]. To our knowledge, SD92-41 is the first C1 subgenotype reported in Chinese mainland, which showed high sequence similarity to the strain 9718-TX-89 from U.S. in 1989, but there is no direct evidence supporting the association with the American strain. This study proves that the C1 gene subtype also appeared in Chinese mainland, but it is not clear whether it is an imported or a local epidemic strain. With sufficient information from retrospective studies, the source of the SD92-41 strain will be identified, and the prevalence of EV-A71 in Chinese mainland before 1998 will be clearer.

Declarations

Ethical Standards:

Animal and Human Rights Statement This study did not involve human experimentation. Biosafety evaluations were approved by the National Institute for Viral Disease Control and Prevention, China CDC. All experimental operations were handled following the Standard Operational Protocol approved by China CDC.

Competing interests

The authors declare no competing interests.

Author Contributions

Conceptualization, Fenfen Si, Dongmei Yan; formal analysis, Fenfen Si, Dongyan Wang, Tianjiao Ji, Yong Zhang, Shuangli Zhu, Junhan Li, Wenbo Xu, Dongmei Yan; Writing—original draft preparation, Fenfen Si; Writing—review and editing, Dongmei Yan; Project administration,Dongmei Yan; funding acquisition, Dongmei Yan. All authors have read and agreed to the published version of the manuscript.

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Data availability:

Whole genome nucleotide sequences for the strain determined in this study have been deposited in the GenBank nucleotide sequence database under accession numbers MW473684.

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Figures

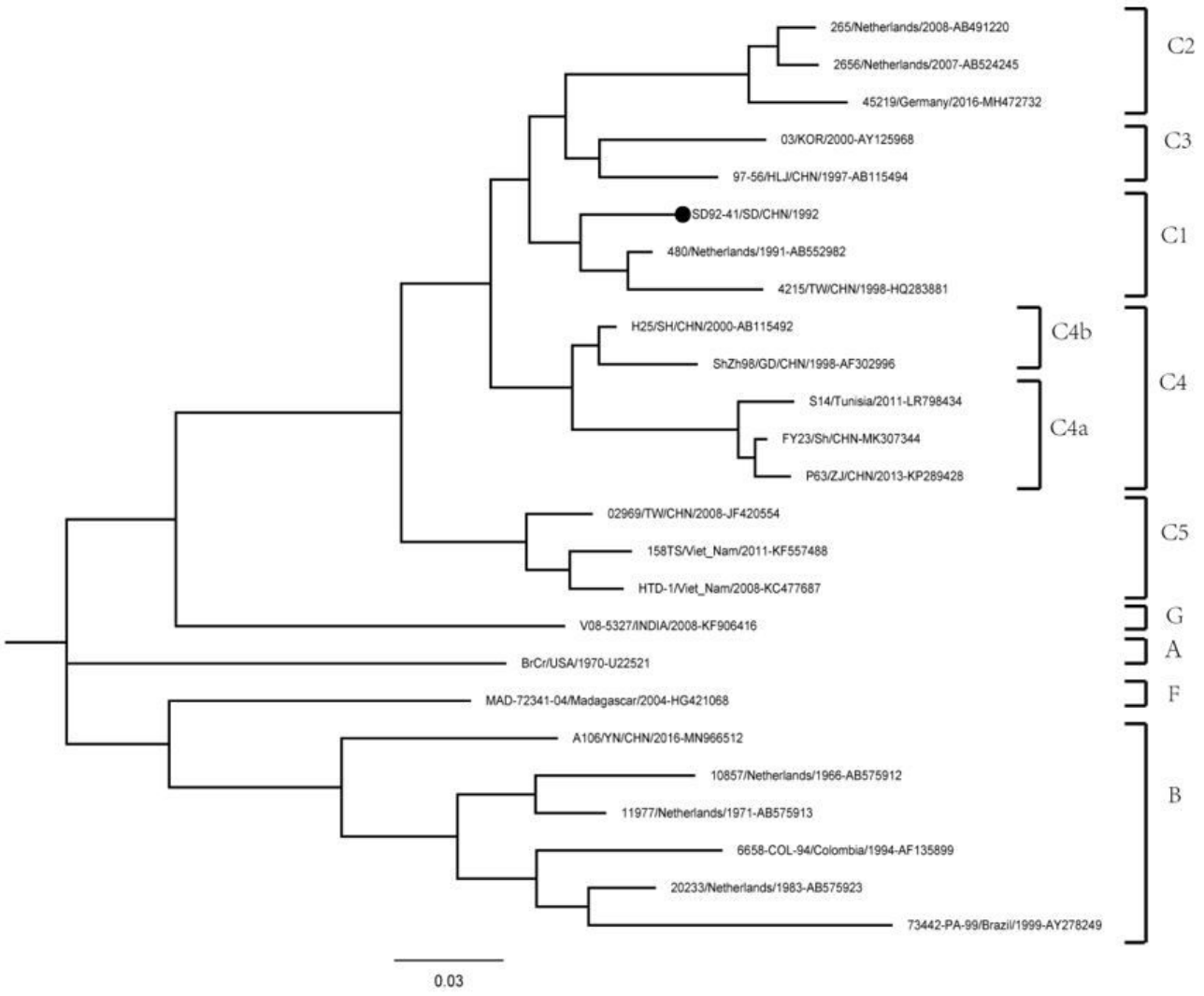

Figure 1

Phylogenetic Tree Based on Entire VP1 Coding Regions of EV71. Note: The SD92-41 strain is marked with a black solid circular.

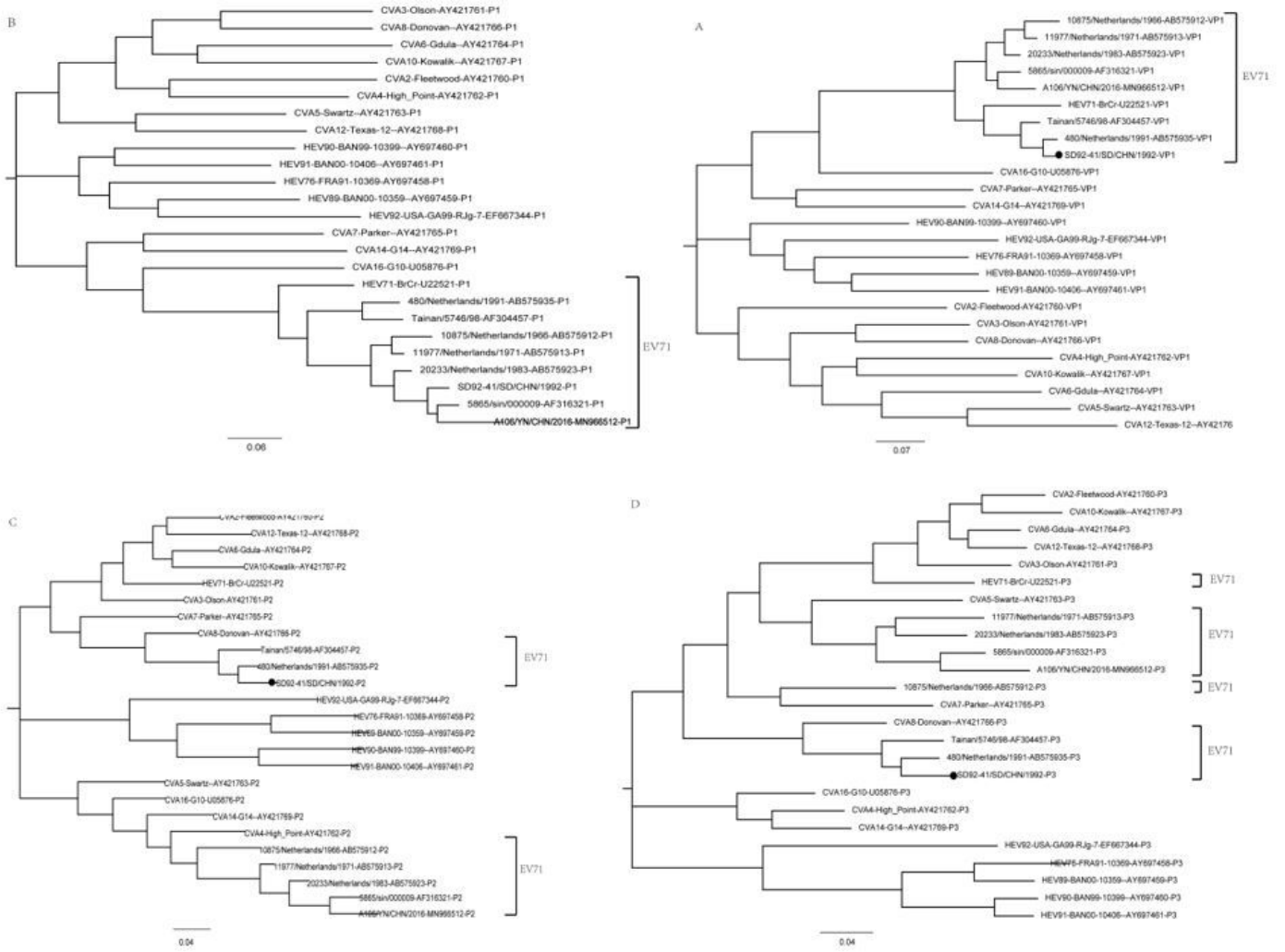

Figure 2

Phylogenetic Tree Based on the VP1, P1, P2, and P3 sequences of EV-A. Note: Maximum likelihood trees were constructed using the GTR + G model and were implemented in MEGA7.0 with 1000 bootstrap replicates. The close circle represents the SD92-41 strain. The scale bars indicate the genetic distance. (a) VP1 coding sequence; (b) P1 coding sequence; (c) P2 coding sequence; (d) P3 coding sequence.

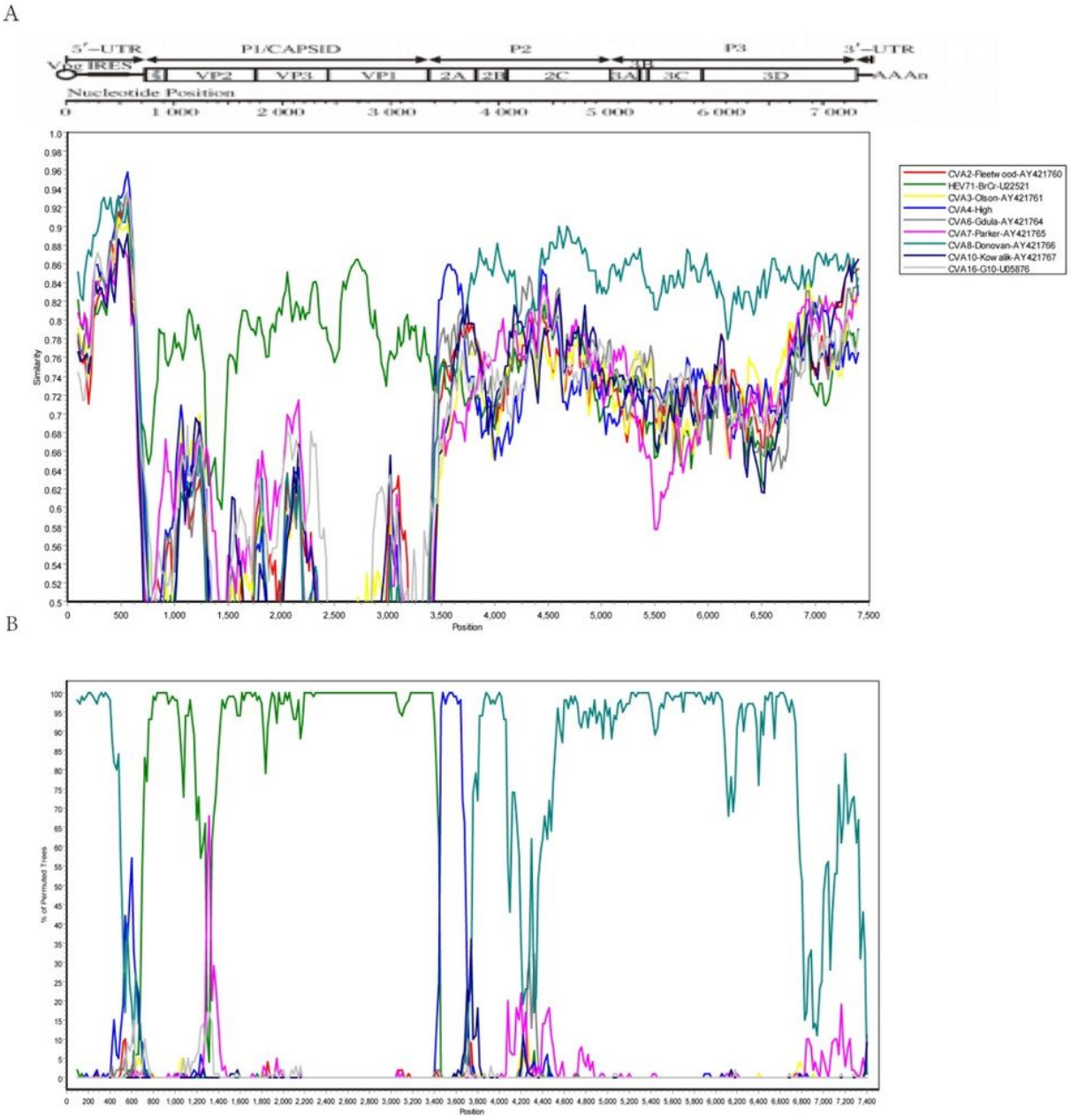

Figure 3

Similarity and Bootscanning Ananlysis of the SD92-41 Strain and Other EV-A Strains. The SD92-41 strain was used as a query sequence.