Research Highlight

Molecular Epidemiology of Enteroviruses Associated with Hand, Foot, and Mouth Disease in the Mainland of China*

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Hand, foot, and mouth disease (HFMD) is a common contagious illness which occurs worldwide both sporadically and in epidemics. The disease mainly affects children and the typical symptoms, which may resolve spontaneously, include mucocutaneous papulovesicular lesions on the hands, feet, mouth, and buttocks. In rare cases, however, the patients may also develop neurological complications such as neurogenic pulmonary edema, aseptic meningitis, and acute flaccid paralysis[1]. The most common etiological agents of HFMD are human enterovirus 71 (EV-A71) and coxsackievirus A16 (CVA16), which have different incidence rates[2]. However, other enteroviruses (EVs) of the EV-A and EV-B species may co-circulate and account for a sizeable proportion of the HFMD pathogen spectrum[3-4].

Since the large-scale outbreaks of HFMD occurred in Linyi City, China in 2007[5], close attention has been paid to HFMD and the disease has been prescribed as a notifiable infectious disease in class C by the Ministry of Health of China. A HFMD virological surveillance system was set up in 2008, which is crucial for monitoring prevalent EV serotypes associated with HFMD[6-7]. With this surveillance system, pathogen detection has focused on EV-A71 and CVA16. Thus, information on other EVs, including their geographical distribution and epidemiological profiles, is limited.

Circulation of EVs associated with recent large outbreaks of HFMD in China highlights the need to understand the molecular epidemiology of EVs, including EV-A71 and CVA16. Sequence analysis of the complete *VP1* region is considered the most reliable and rigorous method to determine EV genotype for molecular epidemiology studies[8].

Phylogenetic classification based on the complete EV-A71 *VP1* region (891 bp) has been used for determining genotype, and this classification has proved to be useful in tracking the genotypes of EV-A71-associated HFMD outbreaks at different times and geographical areas. Based on a molecular epidemiology study of EV-A71 by Brown and colleagues, three genotypes of EV-A71 (A, B, and C) have been recognized[8]. The EV-A71 prototype strain (BrCr strain) is the sole member of the genotype A. Presently, the genotype B contains five subgenotypes (B1-B5), and the genotype C consists of an additional five subgenotypes (C1-C5)[5]. Phylogenetic analysis based on *VP1* sequence indicated that Chinese EV-A71 strains, which have been continuously circulating in China since the first reported detection in Shenzhen City in 1998, belong to the subgenotype C4[5]. The molecular epidemiology of EV-A71 in the mainland of China during the past 15 years reflects the pattern of endemic circulation of subgenotype C4 viruses. Two circulation stages of EV-A71 were observed in the mainland of China from 1998 to 2013. In the first stage (1998-2004), the circulating EV-A71 belonged to the evolutionary branch C4b. After 2004, it was replaced by EV-A71 of the evolutionary branch C4a, which has become the predominant virus circulating in the mainland of China in recent 10 years[9].

Phylogenetic analysis of complete *VP1* sequences has identified two CVA16 genotypes: A and B. The CVA16 prototype strain (G-10 strain) is the sole member of the genotype A. Genotype B CVA16 strains continue to circulate worldwide and at least two subgenotypes (B1 and B2) have been identified. All Chinese CVA16 strains belong to the subgenotype B1, which has been continuously circulating in a wide geographical area of the mainland of China since the first reported detection in Shenzhen City in 1999. Data from the present study indicate that the molecular epidemiology of CVA16 in China during the past 15 years reflects the pattern of endemic circulation of subgenotype B1 viruses, as CVA16 strains of evolutionary branch B1a and B1b are all the predominant viruses that have being circulated in the mainland of China from 1999 to 2013[10].

Similar to EV-A71, CVA16 strains have a wide geographic distribution, but have undergone less genetic changes compared with EV-A71. The rate of CVA16 evolution within a lineage was estimated to be 0.91×10^{-2} substitutions per synonymous nucleotide per year, which was slightly lower than the substitution rate calculated for EV-A71 (1.35×10^{-2})[10]. The same thing in the evolution of EV-A71

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and CVA16 is that their prototype strains are the sole members of genotype A, which gave way to modern strains via molecular evolution. EV-A71 was first identified in 1970 in USA, then it appeared as two co-circulating genotypes (genotypes B and C) during 40 years of evolution. In contrast, the evolution rate of CVA16 is relatively slow despite being first identified in 1951 in South Africa, and all CVA16 strains except the prototype form a single genotype (genotype B) containing two subgenotypes (B1-B2) after more than 60 years of evolution[10].

In addition to EV-A71 and CVA16, other human EVs such as CVA10 and CVA6, which are frequently detected in the specimens collected from HFMD patients, also account for sporadic cases and occasional outbreak of HFMD[3]. However, genetic diversity and molecular evolution of other EVs have not been fully described, unlike those of EV-A71 and CVA16. Enhanced EV surveillance is necessary to predict the potential of these strains in causing outbreaks, as well as to identify the composition of the HFMD pathogen spectrum and epidemic pattern of EVs. Thus, molecular detection for HFMD diagnosis and detailed laboratory-based surveillance of HFMD should be improved in the mainland of China.

The national EV surveillance system is an essential and effective for identifying emerging and re-emerging EV outbreaks and for planning and executing public health interventions. Therefore, continued surveillance of EV circulation in China should not only focus on EV-A71 and CVA16 but also include other EV serotypes. EV surveillance should be enhanced to monitor the emergence of new genetic EV lineages in particular areas that are associated with large-scale outbreaks. Continued monitoring of EVs by both clinical and genetic surveillance should be encouraged.

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