International Journal of Infectious Diseases 87 (2019) 1–7

Contents lists available at ScienceDirect

International Journal of Infectious Diseases

journal homepage: www.elsevier.com/locate/ijid

High proportion of coxsackievirus B3 genotype A in hand, foot and mouth disease in Zhenjiang, China, 2011–2016

Xuemin Fu[a], Lingxiang Mao[b], Zhenzhou Wan[c], Rong Xu[a], Yingying Ma[a], Li Shen[d], Xia Jin[e], Chiyu Zhang[a,*]

[a] Pathogen Discovery and Big Data Center, CAS Key Laboratory of Molecular Virology & Immunology, Institut Pasteur of Shanghai, Chinese Academy of Sciences, University of Chinese Academy of Sciences, Shanghai, China
[b] Department of Clinical Laboratory, Affiliated People's Hospital, Jiangsu University, Zhenjiang, China
[c] Medical Laboratory of Taizhou Fourth People's Hospital, Taizhou, China
[d] Zhenjiang Center of Disease Control and Prevention, Jiuhuangshan South Road, Zhenjiang, China
[e] Viral Disease and Vaccine Translational Research Unit, CAS Key Laboratory of Molecular Virology & Immunology, Institut Pasteur of Shanghai, Chinese Academy of Sciences, Shanghai 200031, China

ARTICLE INFO

Article history:
Received 22 March 2019
Received in revised form 13 July 2019
Accepted 15 July 2019
Corresponding Editor: Eskild Petersen, Aarhus, Denmark

Keywords:
Coxsackievirus B3
Genotypes, hand, foot and mouth disease
Children
Zhenjiang
Myocarditis
Global Epidemic

ABSTRACT

Background: Hand, foot and mouth disease (HFMD) is usually caused by EVA71 and CVA16 except for a few cases that are caused by non-EVA71 non-CAV16 enteroviruses. Coxsackievirus B3 (CVB3) is mostly associated with myocarditis, occasionally with HFMD.

Methods: The partial VP1 gene of enteroviruses were amplified and sequenced from 610 throat swabs from clinically confirmed HFMD children. All available CVB3 near full-length genomic and VP1 sequences were downloaded from GenBank. Phylogenetic and distance analyses were performed using MEGA 7.0.

Results: A total of 238 partial VP1 sequences were obtained, including 93 EVA71 (39%), 79 CAV16 (33%), 29 CVB3 (12%), 24 CVA6 (10%), and 13 other enterovirus serotypes (5.5%). CVB3 is classified into seven genotypes A–G according to phylogenetic and distance analyses. All CVB3 strains from Zhenjiang belonged to genotype A. In contrast to other genotypes that are prevalent in Europe and other regions of China, and often associated with aseptic meningitis and myocarditis, CVB3 genotype A strains identified in Zhenjiang were only detected among HFMD patients.

Conclusions: This high prevalence of CVB3 genotype A among HFMD children has never been reported. This phenomenon has revealed a new epidemic trend of CVB3 among HFMD in China, and it has epidemiological implications for monitoring the epidemic risk of CVB3.

Introduction

Coxsackievirus B3 (CVB3) is a pathogenic enterovirus that belongs to the genus Enterovirus of the family Picornaviridae (Lukashev and Vakulenko, 2017). It was previously divided into five genotypes by alphabetical (A–E) or Roman numeral (I–V) orders (Chu et al., 2010; Laxmivandana et al., 2016; Calderon et al., 2016; Tao et al., 2012; Tian et al., 2014; Wong et al., 2011), but lacked supporting evidence from both phylogenetic and distance analyses that are prerequisites for the genotyping of viruses (Smith et al., 2014). CVB3 has a global distribution and is one of the most common pathogens causing aseptic meningitis, encephalitis, myocarditis and acute flaccid paralysis (Henke et al., 2003; Kim and Nam, 2010); it can result in serious complications especially in neonates and immunocompromised patients (Bendig et al., 2003; Spanakis et al., 2005). The pathogenesis of CVB3 in viral myocarditis was well demonstrated in various mouse models (Garmaroudi et al., 2015).

Hand, foot and mouth disease (HFMD) is a common febrile illness of early childhood, characterized by fever and skin eruptions on the hands and feet, and vesicles in the mouth (Ooi et al., 2010). HFMD is usually caused by EVA71 and CVA16, and some cases caused by EVA71 can manifest severe central nervous system disorders (Liu et al., 2015). Previously, only a few HFMD cases were reported to be caused by CVB3 infection in China, but the

---

[*] Corresponding author.

E-mail addresses: shirleymin.fu@outlook.com (X. Fu), maolingxiang@aliyun.com (L. Mao), wanlv@126.com (Z. Wan), rxu@ips.ac.cn (R. Xu), yyma@ips.ac.cn (Y. Ma), snowforest1982@163.com (L. Shen), xjin@ips.ac.cn (X. Jin), zhangcy1999@ips.ac.cn (C. Zhang).

https://doi.org/10.1016/j.ijid.2019.07.012

proportion of each viral species among HFMD patients had undergone dynamic change over the past few years (Tian et al., 2014; Yao et al., 2015). In particular, a high prevalence of CVB3 was recently observed among HFMD patients in the Jiangsu province of China (Yao et al., 2015).

We previously reported the prevalence of EVA71 and CVA16, as well as non-EVA71 non-CAV16 enterovirus, in children with HFMD in Zhenjiang city, Jiangsu province, during 2011–2016 using RT-qPCR with specific or universal primers (Mao et al., 2018). In this study, we further analyzed the genotype distribution of CVB3 among children with HFMD in a different city of the same Chinese province, and discovered a high prevalence of CVB3 genotype A during 2011–2016.

Materials and methods

Symptoms and definition of HFMD

The HFMD cases were clinically diagnosed according to the guidelines issued by the National Health Commission of China. In brief, HFMD cases were clinically confirmed if a patient had maculopapular or vesicular rash on the hand, foot, mouth, and buttocks with or without fever. Severe HFMD cases were defined as having neurological complications (e.g. aseptic meningitis, encephalitis, acute flaccid paralysis, pulmonary oedema or cardiorespiratory failure).

RNA extraction and amplification

A total of 610 throat swabs were subjected to the amplification and sequencing of the partial VP1 fragment. Viral RNA was extracted from 140 μl swabs using TRIzol^{TM} LS Reagent (Thermo Fisher Scientific, Waltham, MA, USA). RNA was reverse transcribed into cDNA by the SuperScript III reverse transcriptase kit (Invitrogen, Carlsbad, CA, USA). The partial VP1 gene fragment (2570–2872 nt in EVA71 Brcr strain, accession number U05876) was amplified using a nested-PCR assay (GenStar Co., Ltd, Beijing, China) with universal primers described previously (Nix et al., 2006). The amplicon was sent to Shanghai Sunny BioTechnologies Co., Ltd for bidirectional DNA sequencing. A total of 238 partial VP1 sequences were obtained from 610 samples. Of them, 202 sequences are available on GenBank with the accession numbers MK291925-MK291948 and MK604459 for CVB3, MK321960-MK322046 for EVA71, MK322317-MK322327 for CVA6, and MK322328-MK322407 for CVA16. Other sequences are pending assignment of GenBank accession numbers.

There were 372 samples that failed to generate partial VP1 sequences, including 65 with too low concentration of amplicon for Sanger sequencing, 41 with double/mixed peaks in sequencing chromatograms and 266 failures in VP1 amplification. High failure rate in amplification was mainly ascribed to low virus titer or low quality by long-term storage or freezing and thawing.

Phylogenetic analysis

All 63 near full-length CVB3 genomic sequences available in GenBank were downloaded on 3 February, 2019, and 61 with a length of more than 5000 nt were used in the phylogenetic analysis of genomic sequences. The VP1 sequences from 63 full-length genomic sequences were subjected to the phylogenetic analysis of VP1 gene, together with 12 additional VP1 sequences from Shandong, Taiwan and Zhenjiang of China. All available partial VP1 sequences of CVB3 in GenBank were downloaded by the end of October 2018 and used in the phylogenetic analysis with the

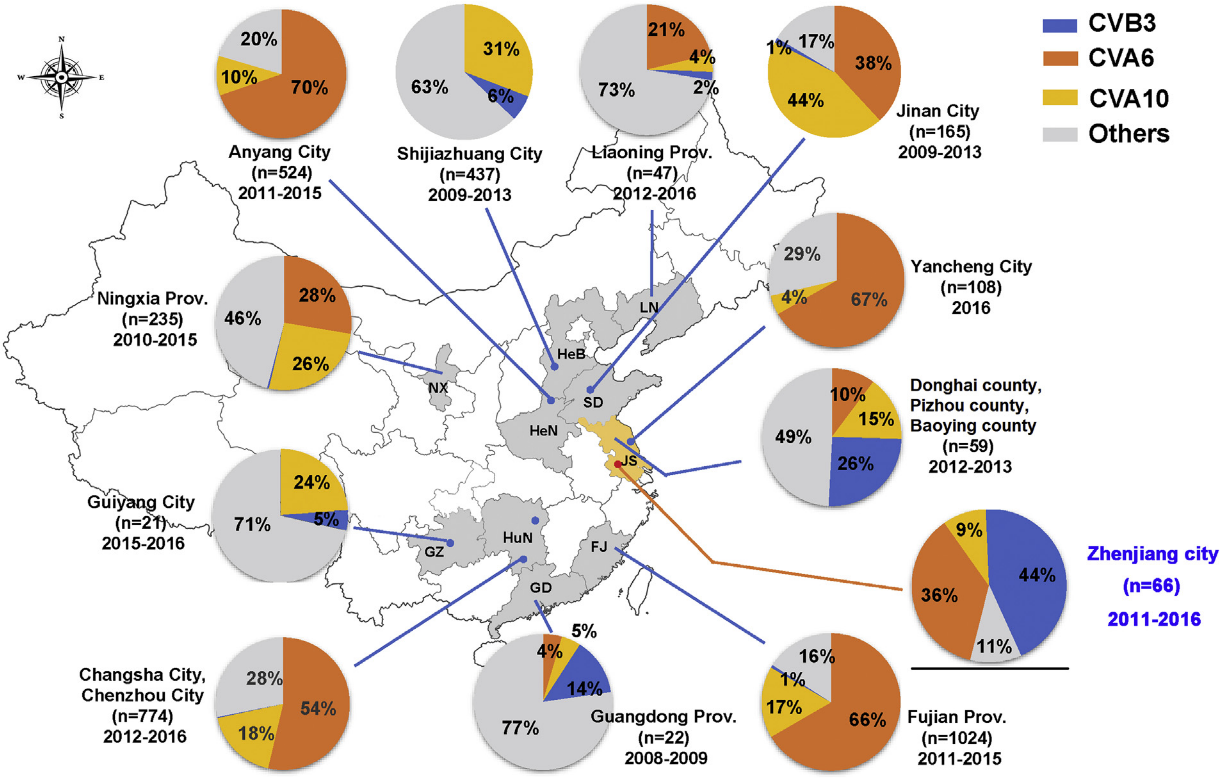

Figure 1. Geographic distributions of CVB3 and other non-EVA71 and non-CVA16 enteroviruses in China. All genotype data were based on the VP1 sequence analyses. The CVB3 strains in Zhenjiang belonged to genotype A, while strains from Shijiazhuang city in Hebei province and three counties in Jiangsu province belonged to genotype G. The genotypes of CVB3 from other regions were not determined because of a lack of available sequences. JS: Jiangsu, LN: Liaoning, HeB: Hebei, SD: Shandong, HeN: Henan, NX: Ningxia, HuN: Hunan, FJ: Fujian, GZ: Guizhou, GD: Guangdong; Prov.: Province. The sources of the data are listed in Supplementary Table S1.

sequences from Zhenjiang. The maximum likelihood (ML) tree was constructed using MEGA7.0 with 1000 bootstrap replications. There were 317 partial VP1 sequences, including 29 CVB3 sequences from Zhenjiang. All Zhenjiang sequences belonged to genotype A. Because of shorter sequences (about 300 nt), five Zhenjiang sequences were excluded from the ML tree analysis. In addition, to minimize the calculation during the ML tree construction, if there are two or more sequences of CVB3 genotypes B–G having higher genetic similarity of more than 99%, only one sequence was kept (i.e. with a genetic distance of less than 1%). The total number of high-similarity sequences was labeled following the name of each representative sequence. After removing, 202 CVB3 sequences were subjected to the ML tree construction. The removing process was performed using Python scripts.

Results

Detection of CVB3 among HFMD children in Zhenjiang

To characterize the genotype diversity of HFMD-associated enteroviruses in this cohort, 610 throat swabs that were previously detected as positive for enteroviruses (including 173 EVA71, 200 CVA16, and 237 other enteroviruses) were selected for the amplification and sequencing of the partial VP1 fragment. A total of 238 partial VP1 sequences were obtained, including 93 from EVA71, 79 from CVA16, and 66 from non-EVA71 and non-CAV16 enteroviruses.

To classify the genotypes of the above enteroviruses, a phylogenetic analysis was performed. Ninety three EVA71 (39%), 79 CAV16 (33%), 29 CVB3 (12%), 24 CVA6 (10%), and 13 other enterovirus serotypes (5.5%) were identified. This high proportion of CVB3 among HFMD children was less likely due to preferential amplification for CVB3 because the universal primers bind to complementary sequences on all five main enteroviruses (Supplementary Figure S1), albeit we have not yet performed experiments to formally exclude this possibility. Among non-EVA71 and non-CVA16 enteroviruses, CVB3 was the most common one, accounting for 44%. This is distinct from HFMD patients in other regions of China where either CVA6 or CVA10 were the 3rd most common enteroviruses following EVA71 and CVA16 (Li et al., 2018) (Figure 1).

Among the 29 CVB3 sequences, 20 were obtained from HFMD boys and 9 from girls, with a male to female ratio of 2.2. The seasonal distributions showed that CVB3 positive cases peaked in August in Zhenjiang (Figure 2).

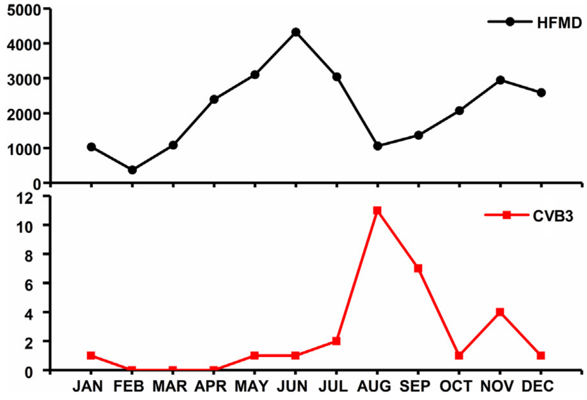

Figure 2. The seasonal distributions of HFMD and CVB3 positive cases in Zhenjiang, 2011–2016.

Comparison of CVB3 prevalence in China

In the Chinese national virological surveillance system, all HFMD cases in China are required to be tested for EVA71 and CVA16 using specific RT-qPCR assays. The genotyping of the other enteroviruses is not mandatory and the method of choice can be Sanger sequencing plus phylogenetic analysis (Oberste et al., 1999). To characterize the overall profile of CVB 3 prevalent in China, we retrieved all available genotyping data of non-EVA71 and non-CVA16 enteroviruses, and compared the proportion of CVB3 among these non-EVA71 and non-CVA16 enteroviruses in different regions. The proportion of CVB3 varied from 0% to 14% in other provinces, all significantly lower than that in Zhenjiang city (43%) ($P < 0.05$) (Figure 1). Furthermore, CVB3 accounted for 26% of non-EVA71 and non-CVA16 enteroviruses in three northern counties of Jiangsu, but it was not found in another northern city, Yancheng, of the same province (Figure 1).

Phylogenetic classification of CVB3

In principle, a combination of phylogenetic and distance analyses should be used for the genotyping of viruses (Smith et al., 2014), and enterovirus genotypes should be equidistant from one another at the genomic level or at least in the VP1 gene (Lukashev et al., 2018). To clearly define the genotypes of CVB3, we constructed maximum likelihood (ML) trees using all available near full-length genomic sequences of CVB3 and their VP1 sequences with an additional 12 VP1 sequences from Taiwan and Zhenjiang of China, Germany and Indonesia (Figure 3). The VP1 tree showed that CVB3 was genetically divided into seven clades with bootstrap values of 98–100%, and defined as genotypes A to G (Figure 3A and Supplementary Figure S2), in keeping with the classifications of EVA71 and CVA16. Except for genotype D which lacks available full-length genomic sequences, all genotypes are supported by the phylogeny of full-length genomic sequences (Figure 3B). The classification of CVB3 was further supported by the distance analysis that showed mean genetic similarities of 89.2–99% within genotypes and 74.6–81.5% between genotypes (Table 1). We then characterized the genetic diversity among CVB3 strains circulating in Zhenjiang by reconstructing the phylogeny of CVB3 with all available partial VP1 sequences from GenBank. All Zhenjiang CVB3 sequences shared 98–100% identity and fell into genotype A, while a vast majority (90%) of CVB3 strains from other regions of China fell into genotype G (Figure 4). Amino acid sequence analysis showed that there are 20, 24 and 19 amino acid differences between genotypes A and G in P1, P2 and P3 proteins, respectively (Supplementary Figure S3).

Global characteristics of CVB3 diversity and epidemiology

CVB3 had been detected among patients with meningitis, encephalitis and myocarditis, and mostly of genotypes G and C (Table 2). In Asia, most CVB3 cases reported in China were genotype G, whereas in Europe most CVB3 strains belonged to genotype C with a small proportion of genotype G (Table 2 and Figure 4). Other than being associated with heart or neurological diseases, CVB3 was also detected among mild HFMD patients, especially in China (Table 2), and most strains were genotype G. Interestingly, we found that all CVB3 strains among HFMD patients in Zhenjiang were genotype A (Figure 4), distinct from that in other countries or regions.

Discussion

Despite the fact that CVB3 has been associated with various diseases, whether its genotypes co-segregate with any unique

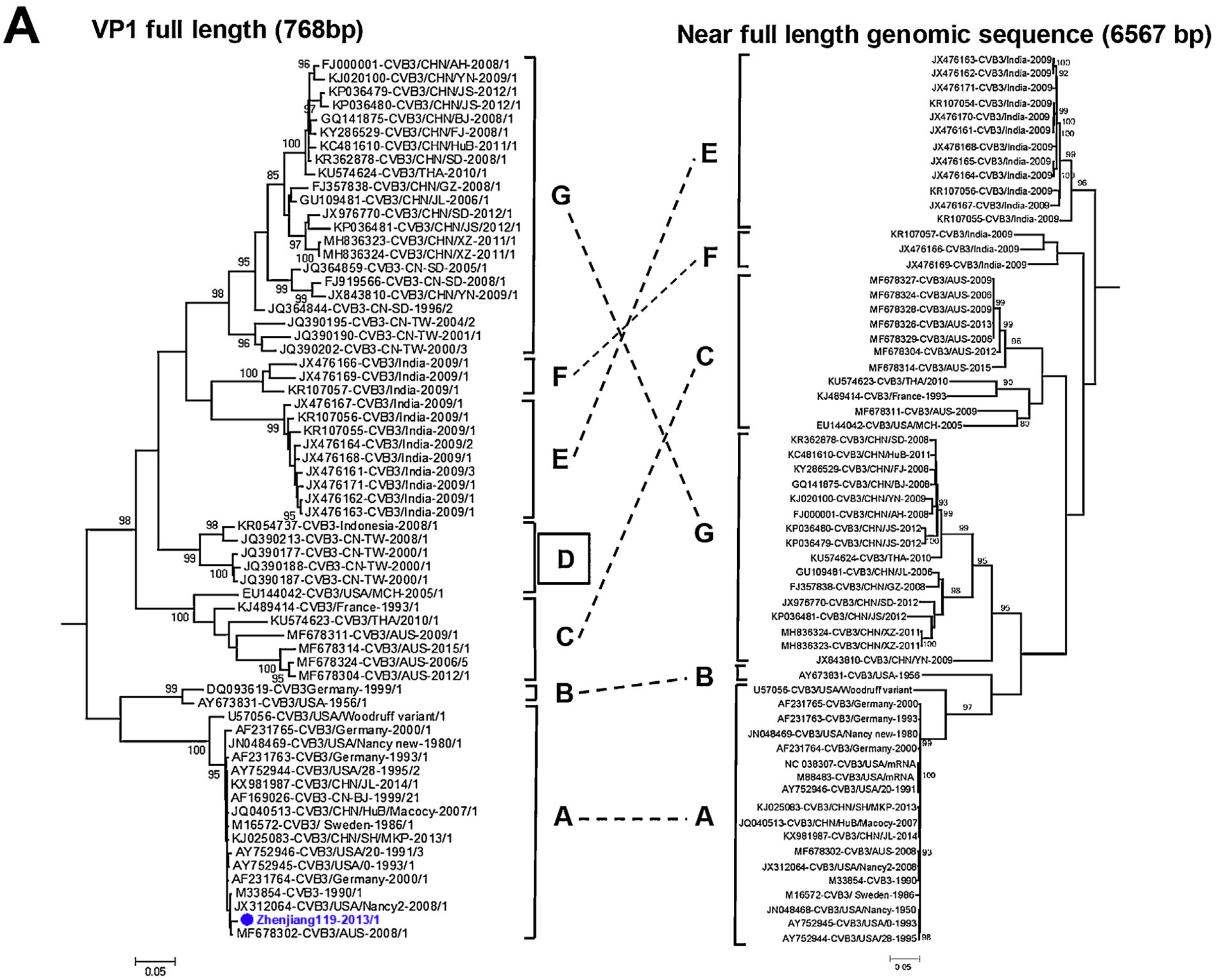

Figure 3. Genotype classification of CVB3 based on VP1 and near full-length genomic sequences. All 63 near full-length genomic sequences available in GenBank were downloaded on January, 2019, and 61 with a genomic sequence of more than 5000 nt were used in the phylogenetic analysis. All 63 full-length genomic sequences were subjected to the phylogenetic analysis of VP1 gene, together with 12 additional VP1 sequences from Shandong, Taiwan and Zhenjiang of China. After moving 10 identical sequences, there were 65 sequences shown in the ML tree of VP1 gene. The ML tree of all available VP1 sequences from GenBank is shown in Supplementary Figure S1.

Table 1
Genetic similarity and distance among seven genotypes of CVB3.

Genotypes	A	B	C	D	E	F	G
A	99.0% (0.010 ± 0.001)						
B	79.6% (0.204 ± 0.017)	96.1% (0.039 ± 0.007)					
C	74.6% (0.254 ± 0.019)	75.4% (0.246 ± 0.017)	89.2% (0.108 ± 0.009)				
D	76.0% (0.240 ± 0.018)	76.3% (0.237 ± 0.016)	79.3% (0.207 ± 0.0170)	94.1% (0.059 ± 0.006)			
E	74.7% (0.253 ± 0.022)	76.6% (0.234 ± 0.017)	78.5% (0.215 ± 0.015)	79.4% (0.206 ± 0.017)	98.1% (0.019 ± 0.002)		
F	75.1% (0.249 ± 0.019)	76.1% (0.239 ± 0.018)	78.9% (0.211 ± 0.015)	80.2% (0.198 ± 0.016)	81.5% (0.185 ± 0.015)	93.5% (0.065 ± 0.008)	
G	74.7% (0.253 ± 0.017)	77.7% (0.223 ± 0.017)	78.3% (0.217 ± 0.013)	79.7% (0.203 ± 0.015)	80.3% (0.197 ± 0.015)	80.9% (0.191 ± 0.014)	91.7% (0.083 ± 0.006)

Note: Mean distance plus standard error are shown in parentheses. The analyses were performed using MEGA 7.0.

disease has been previously unknown. In this study, we phylogenetically classified CVB3 into seven genotypes (A–G) based on both phylogenetic and distance analyses, and reported a high prevalence of CVB3 genotype A among HFMD patients in Zhenjiang, China, during 2011–2016. Distinct from genotype G in other regions of China and genotypes G and C in other countries and causing aseptic meningitis and myocarditis, CVB3 genotype A strains identified here were only detected among HFMD patients with mild symptoms. The results provide new insights into the epidemiology and evolution of CVB3.

A high prevalence of CVB3 has been repeatedly found in Chinese patients with HFMD (Tian et al., 2014; Yao et al., 2015), especially those of genotype A. Of the five earlier CVB3 genotype A strains identified, three were isolated from patients with

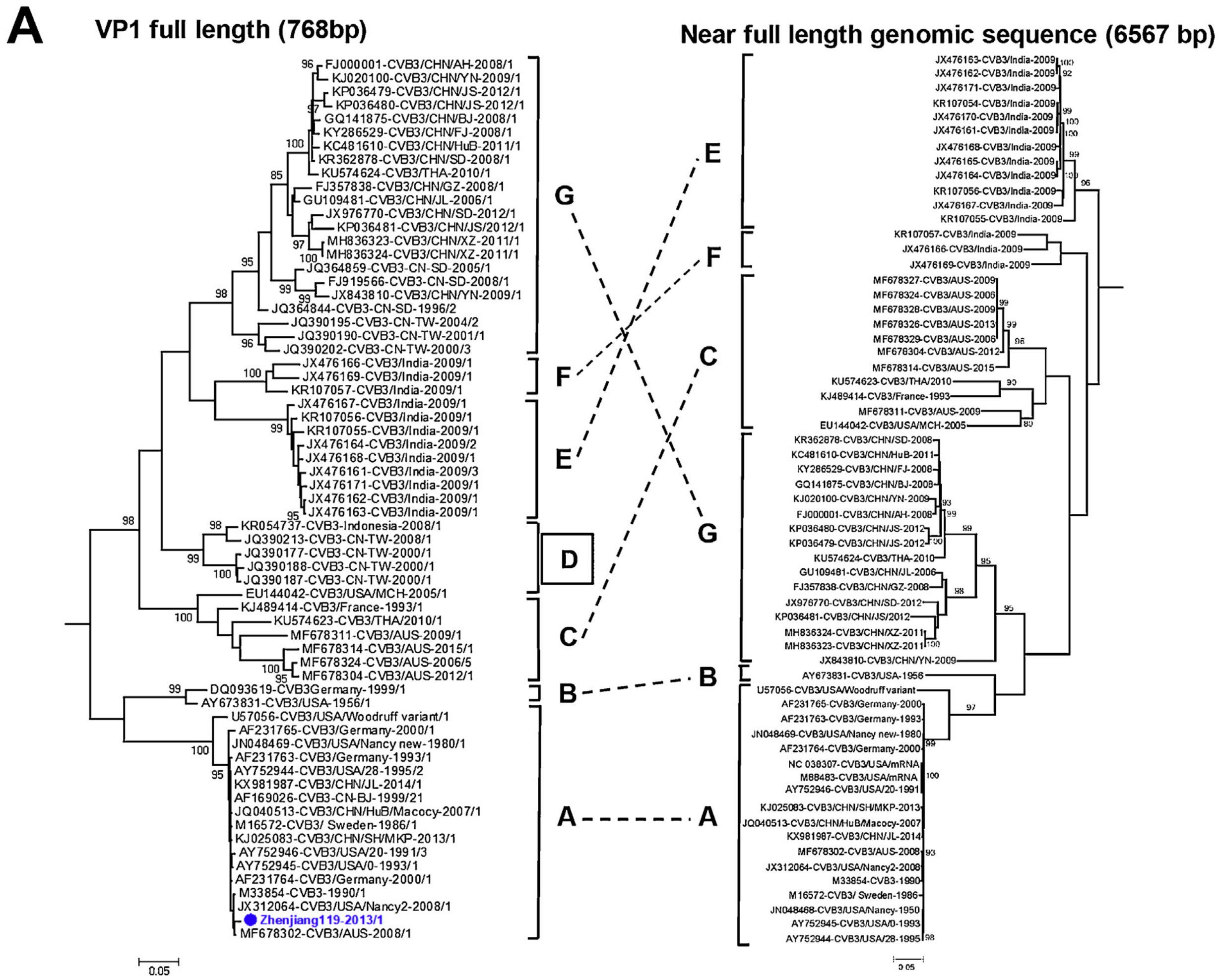

Figure 4. Phylogenetic tree of all available CVB3 partial VP1 sequences from GenBank. To minimize the calculation during the ML tree construction, only one representative sequence was kept if there were two or more sequences with a genetic distance of less than 1%. A total of 202 sequences are shown in the ML tree. The number of high-similarity sequences was labeled after the name of each representative sequence. The strains from Zhenjiang are highlighted by blue, and the strains from other regions of China are labeled by colored triangles. The strains from other countries are highlighted by arrows.

myocarditis or central nervous system disease. The other two genotype A strains identified in Beijing and Heilongjiang were not known to have an association with HFMD. In contrast, the majority (over 80%) of the genotype G strains were isolated from patients with aseptic meningitis or myocarditis, and only a few were from HFMD patients (Chu et al., 2010; Tao et al., 2012, 2014; Wong et al., 2011). These results indicated that the CVB3 infection is not only a pathogenic factor in heart and neurological diseases, but also in HFMD.

This conclusion is further supported by continuous circulation of genotype A in the study population. The 29 CVB3 genotype A strains detected in Zhenjiang were dispersed roughly evenly during the five years between 2011 and 2016 (1, 4, 8, 4 and 12 for each year). This was associated with a unique seasonal pattern of HFMD epidemic in the same city, in which there were either one or two epidemic peaks in alternate years (Mao et al., 2018). The majority (62.1%, 18/29) of the CVB3 genotype A infections occurred during August and September, whereas the other enteroviruses (e.g. EVA71 and CVA16) peaked at different seasons (Mao et al., 2018).

Gender and age appear to be determinants of susceptibility to enterovirus infection in this study cohort. Similar to the predominance of male subjects to infections by other HFMD-associated enteroviruses (Liu et al., 2015), we obtained 20 CVB3 sequences from boys, but only 9 from girls. The male to female ratio was 2.2, consistent with our previous observation of a slight male predominance in the incidence of HFMD in the same city (Mao et al., 2010), and suggested that males were generally more susceptible to infection by CVB3 and other HFMD-associated enteroviruses than females (Chu et al., 2010; Liu et al., 2015). Patient age ranged from 10 to 63 months (median 35 months), which was substantially higher than that reported by Chu et al. (Chu et al., 2010). Only 4 (13.8%) patients were under the age of one year, again different from the previous observations that the majority (60–80%) of the children with CVB3-related myocarditis was younger than one year old (Chu et al., 2010; Calderon et al., 2016). The major reason might be that myocarditis is more likely to occur before the age of one, whereas HFMD is typical in older children.

Enterovirus infection can cause a broad spectrum of clinical syndromes ranging from HFMD, diarrhea, viral myocarditis, encephalitis, aseptic meningitis and upper respiratory tract infection (Mao et al., 2016). As one of the most common enterovirus strains associated with myocarditis (Henke et al., 2003; Kim and Nam, 2010), CVB3 was found to be responsible for the aseptic meningitis outbreak in Shandong (1996, 2000–2002, 2005, and 2008) and Hong Kong (2005 and 2008), as well as some sporadic cases in other regions of China (Chu et al., 2010; Tao et al., 2012, 2014; Wong et al., 2011; Gao et al., 2016). In this study we report the detection of a high prevalence of CVB3 genotype A among HFMD patients in Zhenjiang city. In contrast to genotype G, which often causes aseptic meningitis

Table 2
Global characteristics of CVB3 diversity and epidemiology.

Continent	Country	Province (city)	Period	Symptoms	Case number[a]	Percentage (number)	Genotype[b]
Asia	China	Beijing	2008	Hospital-acquired acute myocarditis	1	–	NA
		Shandong	1999–2010	Acute flaccid paralysis	72	81.9(59)	G
				Aseptic meningitis		9.7(7)	
				HFMD		2.8(2)	
				Other symptoms		5.6(4)	
			2012	Acute myocarditis	1	–	G
		Yunnan	1997–2004	Acute flaccid paralysis	9	–	G
		Hong Kong	1999–2008	Aseptic meningitis	17	29.4(5)	C/G
				Myocarditis		5.9(1)	
				Other symptoms		64.7(11)	
		Taiwan	1992–2005	Aseptic meningitis	29	13.8(4)	D/G
				CNS involvement		13.8(4)	D
				Other symptoms		72.4(21)	–
			2000–2005	Nonspecific febrile	896	–	NA
				Neonatal sepsis			
		Northern Taiwan	1999–2006	Aseptic meningitis	107	11.2(12)	NA
				Encephalitis		7.5(8)	
				Hand-foot-mouth disease		4.7(5)	
				Other symptoms		76.6(82)	
		Hebei	2010–2012	HFMD[c]	26	–	G
		Liaoning and Shandong	2009–2016	HFMD[c]	3	–	G
		Jiangsu (Zhenjiang)	2011–2016	HFMD[c]	29		A
		Jiangsu (three counties)	2012–2013	HFMD[c]	15	–	G
		Guizhou and Guangdong	2015–2016, 2008–2009	HFMD[c]	4	–	G
	India	All area	2009–2010	Aseptic meningitis	3	–	NA
		South-western area	2009–2010	Acute flaccid paralysis	15	66.7(10)	E/F
				Asymptomatic contacts		33.3(5)	
	Indonesian	–	2006–2009	Undifferentiated febrile	1	–	D
	Thailand	–	1987–1989	Myopericarditis	24	–	NA
Europe	Spain	–	2004–2014	Meningitis	50	36(18)	C/G
				Undifferentiated febrile		26(13)	
				Encephalitis, Meningoencephalitis		16(8)	
				Myocarditis, Pericarditis		8(4)	
				Other symptoms		14(7)	
	Germany	All area	2011	Neonatal encephalitis	1	–	G
		Western area	2000–2005	Meningitis, Encephalitis	7	–	NA
	France	–	2005	Meningitis	5	60(3)	NA
				Myopericarditis		20(1)	
				Sudden infant death syndrome		20(1)	
			2000–2004	Acute myocarditis and pericarditis	65	–	NA
Africa	West Africa		2013–2014	Acute flaccid paralysis	2		NA
North America	United States	–	1983–1998	Mild symptoms	1945	93.3(1815)	NA
				Fatal cases		6.7(130)	

Hand foot and mouth disease: HFMD; NA: No available VP1 sequences.

[a] The number of CVB3 positive samples were derived from the references listed in Supplementary Table S2.

[b] The genotypes CVB3 were determined based on reference sequences shown in Figure 2A.

[c] The CVB3 cases were from the HFMD surveillance system.

and myocarditis, CVB3 genotype A viruses identified in this study were only detected among HFMD patients with mild symptoms. Because only HFMD patients were screened in this study, our data do not provide information on whether CVB3 genotype A was also circulating among patients with aseptic meningitis and myocarditis in Zhenjiang. Given the association of CVB3 infection with aseptic meningitis and myocarditis, HFMD patients should also been monitored closely for the risk of the development of heart or neurological diseases (Henke et al., 2003). Furthermore, CVB3 genotype A differs from genotype G by 20, 24, and 19 amino acids in P1, P2 and P3 proteins, respectively. The potential role of various genotypes in the pathogenesis of CVB3-associated diseases should be considered.

HFMD caused by enteroviruses have evolved to include multiple virus species in China (Lu et al., 2012; Zhang et al., 2015). Our finding of a high prevalence of the CVB3 genotype A in Zhenjiang might suggest a new epidemic trend, if CVB3 genotype A could be found in surrounding cities of Zhenjiang (e.g. Nanjing and Yangzhou). Therefore, a continuous epidemiological surveillance is needed to monitor the epidemiology of CVB3.

Conflict of interest

No potential conflict of interest was reported by the authors.

Funding source

This work was supported by the 133 project of Institut Pasteur of Shanghai, CAS, the TOTAL foundation, the National Science and Technology Major Project of China (2017ZX10103009-002) and Shanghai Sailing Program (16YF1412500).

Ethical approval

The study was approved by the Medical Ethics Committee of Jiangsu University (20160225). Informed consents were obtained from all subjects. In this study, all data analyzed were anonymized.

Authors' contributions

C.Z. conceived and designed the study and drafted the manuscript. X.F. performed experiments and phylogenetic analyses. L.M. and L.S. collected samples and clinical data. L.M., R.X. and Y.M. participated in the experiments. X.F. and Z.W. collected the epidemiological data from literatures. C.Z., X.F. and Z.W. analyzed and interpreted the data. X.J. contributed to critical revision of the manuscript. All authors have read and approved the contents of the final manuscript.

Acknowledgments

We gratefully acknowledge Prof. Zhong Huang, at Institut Pasteur of Shanghai (IPS), CAS for his suggestions on this work, and Zhihang Zheng and Yanpeng Li, at IPS, CAS for their technical support.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.ijid.2019.07.012.

References

Bendig JW, Franklin OM, Hebden AK, Backhouse PJ, Clewley JP, Goldman AP, et al. Coxsackievirus B3 sequences in the blood of a neonate with congenital myocarditis, plus serological evidence of maternal infection. J Med Virol 2003;70:606–9.

Calderon KI, Diaz-de Cerio M, Otero A, Munoz-Almagro C, Rabella N, Martinez-Rienda I, et al. Molecular epidemiology of coxsackievirus B3 infection in Spain, 2004-2014. Arch Virol 2016;161:1365–70.

Chu PY, Ke GM, Chen YS, Lu PL, Chen HL, Lee MS, et al. Molecular epidemiology of Coxsackievirus B3. Infect Genet Evol 2010;10:777–84.

Gao F, Bian LL, Mao QY, Chen P, Yao X, Li JX, et al. An epidemic of coxsackievirus B3 infection in infants and children in Jiangsu Province, China: a prospective cohort study. Arch Virol 2016;161:1945–7.

Garmaroudi FS, Marchant D, Hendry R, Luo H, Yang D, Ye X, et al. Coxsackievirus B3 replication and pathogenesis. Future Microbiol 2015;10:629–53.

Henke A, Jarasch N, Wutzler P. Vaccination procedures against Coxsackievirus-induced heart disease. Expert Rev Vaccines 2003;2:805–15.

Kim DS, Nam JH. Characterization of attenuated coxsackievirus B3 strains and prospects of their application as live-attenuated vaccines. Expert Opin Biol Ther 2010;10:179–90.

Laxmivandana R, Cherian SS, Yergolkar P, Chitambar SD. Genomic characterization of coxsackievirus type B3 strains associated with acute flaccid paralysis in south-western India. J Gen Virol 2016;97:694–705.

Li Y, Chang Z, Wu P, Liao Q, Liu F, Zheng Y, et al. Emerging enteroviruses causing hand, foot and mouth disease, China, 2010-2016. Emerg Infect Dis 2018;24:1902–6.

Liu SL, Pan H, Liu P, Amer S, Chan TC, Zhan J, et al. Comparative epidemiology and virology of fatal and nonfatal cases of hand, foot and mouth disease in mainland China from 2008 to 2014. Rev Med Virol 2015;25:115–28.

Lu QB, Zhang XA, Wo Y, Xu HM, Li XJ, Wang XJ, et al. Circulation of Coxsackievirus A10 and A6 in hand-foot-mouth disease in China, 2009-2011. PLoS One 2012;7:e52073.

Lukashev AN, Vakulenko YA. Molecular evolution of types in non-polio enteroviruses. J Gen Virol 2017;98:2968–81.

Lukashev AN, Vakulenko YA, Turbabina NA, Deviatkin AA, Drexler JF. Molecular epidemiology and phylogenetics of human enteroviruses: is there a forest behind the trees?. Rev Med Virol 2018;e2002.

Mao LX, Wu B, Bao WX, Han FA, Xu L, Ge QJ, et al. Epidemiology of hand, foot, and mouth disease and genotype characterization of Enterovirus 71 in Jiangsu, China. J Clin Virol 2010;49:100–4.

Mao Q, Wang Y, Bian L, Xu M, Liang Z. EV-A71 vaccine licensure: a first step for multivalent enterovirus vaccine to control HFMD and other severe diseases. Emerg Microbes Infect 2016;5:e75.

Mao L, Fu X, Wu J, Shen L, Gu J, Yuan Z, et al. The dynamics of the hand, foot and mouth disease epidemic from 2008 to 2016 in Zhenjiang city, China. Future Microbiol 2018;13:1029–40.

Nix WA, Oberste MS, Pallansch MA. Sensitive, seminested PCR amplification of VP1 sequences for direct identification of all enterovirus serotypes from original clinical specimens. J Clin Microbiol 2006;44:2698–704.

Oberste MS, Maher K, Kilpatrick DR, Flemister MR, Brown BA, Pallansch MA. Typing of human enteroviruses by partial sequencing of VP1. J Clin Microbiol 1999;37:1288–93.

Ooi MH, Wong SC, Lewthwaite P, Cardosa MJ, Solomon T. Clinical features, diagnosis, and management of enterovirus 71. Lancet Neurol. 2010;9:1097–105.

Smith DB, Bukh J, Kuiken C, Muerhoff AS, Rice CM, Stapleton JT, et al. Expanded classification of hepatitis C virus into 7 genotypes and 67 subtypes: updated criteria and genotype assignment web resource. Hepatology 2014;59:318–27.

Spanakis N, Manolis EN, Tsakris A, Tsiodras S, Panagiotopoulos T, Saroglou G, et al. Coxsackievirus B3 sequences in the myocardium of fatal cases in a cluster of acute myocarditis in Greece. J Clin Pathol 2005;58:357–60.

Tao Z, Song Y, Li Y, Liu Y, Jiang P, Lin X, et al. Coxsackievirus B3, Shandong Province, China, 1990-2010. Emerg Infect Dis 2012;18:1865–7.

Tao Z, Wang H, Li Y, Liu G, Xu A, Lin X, et al. Molecular epidemiology of human enterovirus associated with aseptic meningitis in Shandong Province, China, 2006-2012. PLoS One 2014;9:e89766.

Tian H, Zhang Y, Sun Q, Zhu S, Li X, Pan Z, et al. Prevalence of multiple enteroviruses associated with hand, foot, and mouth disease in Shijiazhuang City, Hebei province, China: outbreaks of coxsackieviruses a10 and b3. PLoS One 2014;9:e84233.

Wong AH, Lau CS, Cheng PK, Ng AY, Lim WW. Coxsackievirus B3-associated aseptic meningitis: an emerging infection in Hong Kong. J Med Virol 2011;83:483–9.

Yao X, Bian LL, Lu WW, Li JX, Mao QY, Wang YP, et al. Enterovirus spectrum from the active surveillance of hand foot and mouth disease patients under the clinical trial of inactivated Enterovirus A71 vaccine in Jiangsu, China, 2012-2013. J Med Virol 2015;87:2009–17.

Zhang C, Zhu R, Yang Y, Chi Y, Yin J, Tang X, et al. Phylogenetic analysis of the major causative agents of hand, foot and mouth disease in Suzhou City, Jiangsu province, China, in 2012-2013. Emerg Microbes Infect 2015;4:e12.