Journal of Clinical Virology 70 (2015) 7–13

Contents lists available at ScienceDirect

Journal of Clinical Virology

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

# Molecular epidemiological analysis of Saffold cardiovirus genotype 3 from upper respiratory infection patients in Taiwan

Tsuey-Li Lin [a], Ting-Han Lin [a], Shu-Chun Chiu [a], Yuan-Pin Huang [a], Cheng-Mao Ho [a], Chia-Chi Lee [a], Ho-Sheng Wu [a,b,**], Jih-Hui Lin [a,*]

[a] Center for Research, Diagnostics and Vaccine Development, Centers for Disease Control, Taiwan, Taipei, Taiwan
[b] School of Medical Laboratory Science and Biotechnology, Taipei Medical University, Taipei, Taiwan

ARTICLE INFO

*Article history:*
Received 2 December 2014
Received in revised form 22 June 2015
Accepted 23 June 2015

*Keywords:*
Saffold virus
Upper respiratory infection
Molecular epidemiology
Taiwan

ABSTRACT

*Background:* Saffold cardiovirus (SAFV) belongs to the *Cardiovirus* genus of Picornaviridae family, and may be a relevant new human pathogen; Thus far, eleven genotypes have been identified. The SAFV type 3 (SAFV-3) is thought to be the major genotype and is detected relatively frequently in children with acute gastroenteritis and respiratory illness. The epidemiology and pathogenicity of SAFV-3 remain unclear.
*Objectives:* To investigate the genomic and epidemiologic profiles of SAFV-3 infection in Taiwan.
*Study design:* Virus was detected in respiratory samples from children suffering for URI. SAFV-3 isolates were detected by isolation on cell culture and IF assay. The molecular typing was performed by RT-PCR and was sequenced to compare with reference strains available in the NCBI GeneBank. Serum samples were collected from 2005 to 2013 in Taiwan for seroprevalence investigation.
*Results:* A total of 226 specimens collected from children with URIs, 22 (9.73%) were positive for SAFV-3. The majority of SAFV-3 infections were found in children less than 6 years of age (14 of 22, 63.6%). Genetic analysis of VP1 coding region of Taiwanese isolates shown an 83.2–97.7% difference from other available SAFV-3 sequences in NCBI GenBank. Phylogenetic analysis revealed there is three genetic groups of SAFV-3 co-circulated in Taiwan during the study period. In addition, seroprevalence investigation results indicated that SAFV-3 infection occurs early in life and 43.7–77.8% of children aged between 6 months to 9 years old, had neutralizing antibodies against SAFV-3.
*Conclusion:* SAFV-3 may have circulated in Taiwan for some time and it appears to be one of the etiological agents responsible for URIs in children.

© 2015 Elsevier B.V. All rights reserved.

## 1. Background

Saffold cardiovirus (SAFV) belongs to the *Cardiovirus* genus of Picornaviridae family, which includes other pathogenic viruses involved in human hand, foot and mouth diseases (HFMD) such as enteroviruses [1,2]. The Cardiovirus genus includes two species: *Theilovirus* and Encephalomyocarditis virus. A newly identified Theilovirus named SAFV has been isolated from an 8-month-old child with fever of unknown origin in 2007 and designated as SAFV genotype 1 (SAFV-1) [1,3]. SAFV is distinct from Theiler's murine encephalomyelitis virus (TMEV), Vilyuisk human encephyalomyelitis virus (VHEV) and Theiler-like rat virus (TRV) making it the fourth Theilovirus type. SAFV is a non-enveloped pathogen with a positive sense, single-strand genomic RNA of approximately 8050 nucleotides (nts). All SAFVs identified to date are significantly divergent from the documented animal cardioviruses, especially in the region encoding the structural capsid proteins that are important from receptor binding. The nucleotide sequence of the SAFV isolate showed a strong similarity to TRV, which had been previously isolated from rats in Japan [3,4]. Four capsid proteins (VP1 to VP4) that constitute the monomeric units of the viral icosahedral-shaped capsid [5], and VP1 protein plays a crucial role in cell entry via interaction of an Arg–Gly–Asp (RGD) triplet with integrin on the cell surface [6,7]. The SAFV typing is based on its VP1 gene, providing a reliable locus to type all SAFV strains as described for enteroviruses by Oberste et al. [8]. To date,

* Corresponding author at: Center for Research, Diagnostics and Vaccine Development, Centers for Disease Control, Taiwan. No.161, Kun-Yang St., Taipei 11513, Taiwan. Fax: +886 2 27853944.
** Corresponding author at: Center for Research, Diagnostics and Vaccine Development, Centers for Disease Control, Taiwan. No.161, Kun-Yang St., Taipei 11513, Taiwan. Fax: +886 2 27837779.
*E-mail addresses:* wuhs@cdc.gov.tw (H.-S. Wu), jeffy320@cdc.gov.tw (J.-H. Lin).

http://dx.doi.org/10.1016/j.jcv.2015.06.100
1386-6532/© 2015 Elsevier B.V. All rights reserved.

the majority of SAFV available nucleotide data concerns the VP1 gene, and a total of eleven genotypes have now been identified [9,10]. Of them, SAFV-3 is thought to be the major genotype and is detected relatively frequently in patients with acute gastroenteritis and respiratory illness [11–15]. However, the epidemiological and genetic characteristics of SAFV-3 infection remain ambiguous. In this study, we describe the first SAFV-3 isolate along with its growth characteristics in cell lines, genomic and epidemiological profiles of SAFV-3 infection in Taiwan. These data provide a better understanding of its biology and possible associations with disease and will also contribute to the information on the clinical and epidemiological impact of SAFV-3.

## 2. Objectives

To describe the clinical and epidemiological features as well as the molecular data, we investigate the epidemiologic and genomic profiles of SAFV-3 infection in Taiwan by carry out a seroprevalence survey and molecular analysis of Taiwanese SAFV-3 isolates.

### 2.1. SAFV-3 detection

According to the Communicable Disease Control Act, all severe complicated cases of suspected enterovirus and influenza virus infection must be reported and throat swabs, rectal swabs, stool, cerebrospinal fluid, and serum samples were collected and send to Taiwan CDC through the National Notifiable Disease Surveillance System (NNDSS). The SAFVs used in this study were collected and isolated by Taiwan CDC collaborating laboratories of virology (TCCLV) between February and July 2012, throat swab specimens were obtained from children with symptoms related to enterovirus (EV) infections, which was determined at least one of the following features: fever, headache, myalgia, rhinorrhea, cough, sore throat, vomiting, diarrhea, pharyngeal vesicles or ulcers, paralysis, and skin rash. After collection, specimens were prepared in minimal essential medium (MEM) and centrifuged at 3000 × *g* at 4 °C for 30 min and supernatants were used for virus isolation. Samples were inoculated in the MDCK, MK2, HEp-2, RD, MRC-5, Vero, A549, and HeLa cell lines, and the cell growth were evaluated in MEM containing 2% (v/v) fetal bovine serum and antibiotics in a 5% $CO_2$ humidified incubator for 7–10 days or until cytopathic effects (CPE) being observed. Cultures were tested by respiratory virus and pan-enterovirus indirect immunofluorescence assay (IFA) (Millipore Cat. No. 3105 and Cat. No. 3360) and EV group blends (coxsackievirus blend, echovirus blend, enterovirus 70/71 blend, and poliovirus blend). If the culture was identified as a non-typable enterovirus, a one-step RT-PCR assay was performed to target the VP1 gene segment of SAFV-3. The analytical scheme for the laboratory differentiation of SAFV-3 was shown in Fig. 1.

### 2.2. Seroprevalence investigation

Serum samples were collected from 2005 to 2013; the subjects were randomly sampled by computer-assisted selection from Laboratory Information Management System for diagnosis of suspected EV71 server cases. Only age and clinical symptom of study subjects were recorded, other information on ethic background of individuals was not included. For seroprevalence investigation, neutralization test was performed in RD cell for EV71 and HEp-2c cell for SAFV-3 culture. In brief, Serial two-fold dilutions of serum, from 1:8 to 1:1024 were incubated with 50–100 50% cell infectious doses (CCID50) of the virus for one hour at 36 °C and added to cell culture in 96-well plates. Cytopathic effect of RD cell and HEp-2c cell was accounted 4 days and 7 days respectively after infection and 7 days. Antibody titer of individual sera was expressed as a reciprocal of a dilution that completely neutralized infectivity. Neutralizing titer equal to or greater than 8 was considered as positive.

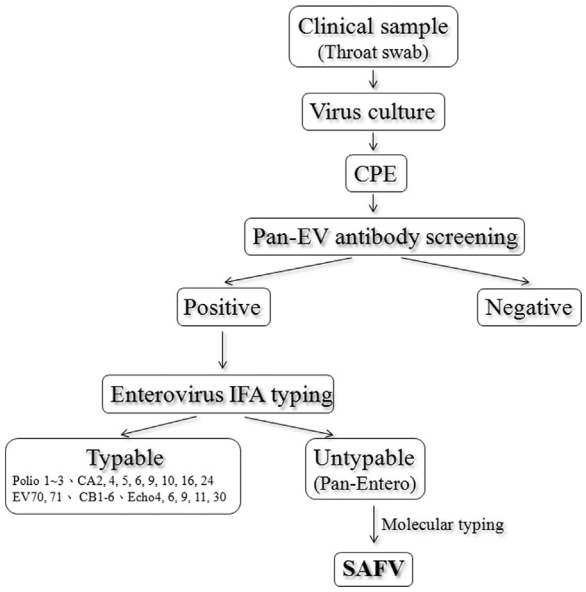


**Fig. 1.** Schematic illustration of SAFV-3 detection workflow.
Sample processing steps included the virus isolation from clinical samples, followed by IFA identification when CPE were observed. The IFA untypable viruses were then detected by RT-PCR and sequencing.

### 2.3. Complete genomes sequencing and phylogenetic analysis

Virus nucleic acids in clinical samples were extracted by using the NucliSens easyMAG system (bioMerieux, Marcy-1′ Etoile, France) according to the manufacturer's instructions. SAFV RNA was detected by using RT-PCR method as previously described [16]. Two sets of primers were used to amplify and sequence the VP1 gene (primer sequences are as follow: SAVF 3-1F 5′-TCA GTA TCG AGG CTC TCT CA-3′, SAVF 3-1R 5′-TTG AAC CAC, ACT GAC GGT AA-3′, SAFV 3-2F 5′-GCA GAA TTC CAT CCT GCT GA-3′, SAFV 3-2R 5′-GGG GAG TTG ACA GGG TTT TT-3′) for phylogenetic analysis. Complete coding sequences were determined according to a previous report [17]. Sequences were first edited manually with MEGA 6 and then aligned with ClustalW [18,19]. Similarity plot for the full genome sequence of SAFV-3 was made using SimPlot (version 3.5.1, http://sray.med.som.jhmi.edu/SCRoftware/simplot/).

The Bayesian Markov Chain Monte Carol (Bayesian MCMC) method implemented in BEAST (v 1.7.4) was used to determine the divergence time, rates of evolution, and molecular clock phylogenies [20]. To determine the optimal substitution model we performed jModelTest [21], and the model best fit our sequence dataset was General Time Reversible (GTR) model with a discretized γ-distribution (GTR + Γ), allowing for nucleotide rates to vary among sites within the protein coding sequence alignments. All nucleotide sequences obtained in this study have been submitted to NCBI with the GenBank accession numbers KR012470-KR012491.

## 3. Results

### 3.1. Virus isolation and identification

The Taiwan CDC collaborating laboratories and sentinels reported a total 1454 case of laboratory-confirmed enterovirus infections in 2013, 226 of them were not able to specific typed

Download English Version:

https://daneshyari.com/en/article/6119820

Download Persian Version:

https://daneshyari.com/article/6119820

Daneshyari.com