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Three clusters of Saffold viruses circulating in children with diarrhea in Japan

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ABSTRACT

Saffold virus (SAFV) is a newly discovered human virus in the genus *Cardiovirus*, family *Picornaviridae*. The virus was first described from fecal specimens of a child with fever of unknown origin in 2007. A total of 454 fecal specimens were collected from children with diarrhea attended clinics in Japan, 2010–2011, 7 (1.5%) were positive for SAFV. Mixed-infections of SAFV and other enteric viruses (rotavirus, norovirus, and bocavirus) were found in four out of seven cases, while monoinfection by SAFV alone was detected in three cases. In addition to diarrhea, fever and vomiting were observed in three children and mild dehydration in one case. No particular symptoms of cough and rhinorrhea were noted. Analysis of partial VP1 nucleotide sequence of 7 Japanese SAFV strains revealed that 5 SAFV sequences were most closely related with SAFV2 reference strains, but separated into SAFV2-A (3 strains) and SAFV2-B (2 strains). In addition, the other two strains were classified as SAFV3. Our results indicated that SAFVs (SAFV2 and SAFV3) were circulated in children with acute gastroenteritis in Japan during 2010 and 2011 epidemic season.

1. Introduction

Gastroenteritis is one of the leading causes of morbidity and mortality in infants and young children worldwide (Wiegering et al., 2011; Dennehy, 2011). The majority of acute gastroenteritis in children causes by virus infections. Among these, rotavirus, calicivirus (norovirus and sapovirus), enteric adenovirus, and astrovirus have been reported as the most important etiologic viral agents (Dennehy, 2011; Chaimongkol et al., 2012). On the other hand, there are several reports of newly discovered enteric viruses, which are potentially associated with acute gastroenteritis in human, including Saffold virus (SAFV). SAFV is a new species of *Cardiovirus* in the *Picornaviridae* family. The virus was isolated and identified recently from fecal specimens of a child with fever of unknown origin in the US (Jones et al., 2007). Several articles have confirmed recently that SAFVs have also been isolated from fecal and nasal specimens collected from patients worldwide (Abed and Boivin, 2008; Blinkova et al., 2009; Xu et al., 2009; Ren et al., 2009; Itagaki et al., 2010; Tsukagoshi et al., 2010; Dai et al., 2011; Khamrin et al., 2011; Chua et al., 2011). Most recently, the virus was also found to be associated with serious invasive infection of the CSF, myocardium, and blood specimens (Nielsen et al., 2012). On the other hand, several molecular epidemiological studies demonstrated that SAFVs have also been detected in asymptomatic controls (Blinkova et al., 2009; Xu et al., 2009). In many studies, SAFV positive subjects showed high co-infection rates with other viral pathogens (Drexler et al., 2008; Chiu et al., 2008; Xu et al., 2009; Dai et al., 2011; Khamrin et al., 2011). Seroepidemiological studies of SAFV demonstrated that most humans acquire SAFV infections in early childhood, indicating that natural infections occur in human populations (Zoll et al., 2009; Galama et al., 2011). Accordingly, it is not clear whether SAFV associates with the diseases, including gastroenteritis in humans because the clinical significance and the epidemiological data of SAFV are limited.

The genome of SAFV is a positive sense single-stranded RNA of approximately 8000 nucleotides (nt) long. The genome consists of four structural viral proteins (VP4, VP2, VP3, and VP1), and seven nonstructural proteins (2A, 2B, 2C, 3A, 3B, 3C, and 3D) (Jones et al., 2007; Chiu et al., 2008; Zoll et al., 2009; Drexler et al., 2010). SAFV comprises of at least 11 genotypes, of which the two new SAFV10 and SAFV11 sequences have recently been deposited on GenBank database (Naeen and Shimizu, unpublished data; www.picornaviridae.com).

In Japan, epidemiological surveillances of SAFV in patients with respiratory tract infection have initially been detected in the

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specimens collected in 2008 (Tsukagoshi et al., 2011), 1 year after the first report of SAFV in 2007. The SAFVs associated with respiratory tract infection in Japan were responsible for about 2% up to 24% and the virus belonged to SAFV2, 3, and 6 genotypes (Itagaki et al., 2010, 2011; Tsukagoshi, 2010, 2011; Himeda and Ohara, 2012). In addition, one SAFV3 had been found in CSF of a patient with aseptic meningitis in 2008 (Himeda et al., 2011). However, epidemiological surveillance of SAFV in children with acute gastroenteritis in Japan is limited. In order to gain the overview genetic background as well as the clinical significance of SAFV circulating in Japan, we conducted the epidemiological study of SAFV in children with diarrhea in Japan during 2010–2011. Sequence and phylogenetic analyses of SAFVs detected in the present study were further characterized for their genetic evolutionary relationships with SAFVs circulating in this area and SAFV reference strains.

2. Materials and methods

2.1. Specimen collection

A total of 454 stool specimens were collected from children with diarrhea attending several clinics, in Japan. The study period was from January 2010 to June 2011. Only the pediatric patients who had a clinical diagnosis of acute gastroenteritis with watery diarrhea have been included in this study. The ages of the patients ranged from neonate up to 6 years old. The stool specimens were also screened for other diarrheal viruses including group A, B, C rotaviruses, adenovirus, norovirus GI and GII, sapovirus, astrovirus, Aichi virus, human parechovirus, enterovirus, and bocavirus based on the protocols described previously (Yan et al., 2003, 2004; Pham et al., 2010).

2.2. SAFV detection and genotype characterization

The presence of SAFV in fecal specimens was detected by RT-nested PCR which targeted the 5′-untranslated region (UTR) of the genome (Drexler et al., 2008). The SAFVs detected in our study were analyzed further by amplification of the viral protein 1 (VP1) gene (Itagaki et al., 2010) and direct sequencing of the VP1 PCR amplicon was performed using BigDye Terminator Cycle Sequencing Kit (Applied Biosystems, Foster City, CA). The sequences of partial VP1 detected in this study were compared with those of reference strains available in the NCBI GenBank database using BLAST server. A phylogenetic tree based on the partial VP1 genome sequence (348 bp, corresponding to nt position 3257–3604 of UC1 (EU376394) reference strain) was constructed by the neighbor-joining method using MEGA (v5.05) software (Tamura et al., 2011). The trees were statistically supported by boot-strapping with 500 replicates. Distances were computed using the maximum composite likelihood method and are in units of numbers of base substitutions per site. All positions containing gaps and missing data were eliminated from the dataset (Complete deletion option).

2.3. Nucleotide sequence accession numbers

The partial nucleotide sequences of Saffold virus VP1 genes described in the present study have been deposited in the GenBank database under the accession numbers JX169782–JX169788. The following SAFV VP1 sequences of reference strains obtained from the GenBank database were used in the phylogenetic analysis; SAFV1:HQ668170, HQ668173, FJ586240, EF165067, GU943516, FJ464766, FJ464777; SAFV2:AB545780, FN999911, EU681176, JN652233, JN652232, JF693617, EU681177, JF693612, EU604749, EU604747, EU604748, EU604750, FJ463601, AB545786, HQ668171, HQ668172, FJ374267, FR682076, GU943518, GU943517; SAFV3:FJ997531, FJ463605, EU604745, EU604746, EU681179, GU943513, AB542807, AB542806, HQ902242; SAFV4:FJ463600, FJ463603, FJ463606; SAFV5:FJ463615, FJ463616; SAFV6:FJ463617; SAFV7:FJ463602; SAFV8:FJ463604; SAFV9:FJ997532.

3. Results

From a total of 454 fecal specimens collected from children with diarrhea in Japan, 7 (1.5%) were positive for SAFV by RT-nested PCR screening method. The positive samples were detected sporadically along the study period in March, April, May, July–October. It was interesting to observe that among 7 SAFV positive samples, double- or triple-infections together with other enteric viruses were found in 4 (57%) pediatric patients, while monoinfection with SAFV alone was detected in 3 (43%) cases. Mixed infections of SAFV with rotavirus, norovirus, and bocavirus were observed in this population (Table 1).

Medical records of all SAFV positive patients were reviewed and clinical characteristics are shown in Table 1. Of these seven children with SAFV positive, the male to female ratio was 3:4. The age ranged from 1 to 6 years, of which SAFV infections were found frequently in children with the age of younger than 2 years. Diarrhea was observed in all patients and the number of diarrheal episodes per day was less than 4 times. Diarrhea in these patients lasted for 3 to 7 days. In seven patients who were positive for SAFV, fever and vomiting were observed in three children and mild dehydration in one case. No particular symptoms of cough and rhinorrhea were noted.

The SAFVs detected in our study were analyzed further by amplification of the partial VP1 region. All PCR products of the partial VP1 were sequenced and compared with those of 9 established SAFV reference genotypes (SAFV1–9). The phylogenetic analysis of the partial VP1 nucleotide sequences (348 nt long) of all seven SAFV strains shown in Fig.1 indicated that SAFVs detected in this study were divided into two genotypes, SAFV2 and SAFV3. Interestingly, phylogenetic evaluation among SAFV2 strains included in this analysis revealed the existence of at least two major lineages, tentatively proposed as SAFV2-A and SAFV2-B. Three strains of SAFV2 (9964/2010, 9957/2010, and 0169/2010) formed exclusively within SAFV2-A lineage. The VP1 sequences of 9964/2010 and 9957/2010 strains were more closely related with each other but less closely related to that of 0169/2010 strain. All these SAFVs showed the close genetic background with the SAFV2-A Yamagata.JPN/2069.09, Nijmegen2008, D/VI2229/2004, S19, S14, and 09T9707DK strains over 84%, while the nucleotide sequence identities with that of SAFV2-B strains were lower than 81%. In addition, it was interesting to observed that the other two SAFV2 strains (9992/2010 and 0032/2010) formed their own cluster with the SAFV Yamagata.JPN/2474.09 within SAFV2-B lineage and exhibited the nucleotide sequence identity ranging from 90.2% to 95.8%, but the nucleotide sequence identities with those of SAFV2-A strains were less than 81%. Additionally, four amino acid differences were observed among partial VP1 sequences of SAFV2-A and SAFV2-B strains found in this study (data not shown).

As shown in Fig. 1, the phylogenetic analysis revealed that the other two SAFV strains (9913/2010 and 0312/2011) detected in this study clustered with the SAFV3 reference strains. These SAFV 9913/2010 and 0312/2011 strains exhibited high level of nucleotide sequence identity greater than 99% with each other and were also closely related to the SAFV3 strains isolated previously in Japan (07-Aichi10247 and JPN08-404) with the nucleotide sequence identity ranging from 99.1% to 99.8%, but exhibited lesser nucleotide sequence identity (less than 73%) with SAFV1–2, and SAFV4–9 genotypes (data not shown).