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Research paper

Genetic diversity and intergenogroup recombination events of sapoviruses detected from feces of pigs in Japan

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ABSTRACT

Sapoviruses (SaV) are enteric viruses infecting humans and animals. SaVs are highly diverse and are divided into multiple genogroups based on structural protein (VP1) sequences. SaVs detected from pigs belong to eight genogroups (GIII, GV, GVI, GVII, GVIII, GIX, GX, and GXI), but little is known about the SaV genogroup distribution in the Japanese pig population. In the present study, 26 nearly complete genome (> 6000 nucleotide: nt) and three partial sequences (2429 nt, 4364 nt, and 4419 nt in length, including the entire VP1 coding region) of SaV were obtained from one diarrheic and 15 non-diarrheic porcine feces in Japan via a metagenomics approach. Phylogenetic analysis of the complete VP1 amino acid sequence (aa) revealed that 29 porcine SaVs were classified into seven genogroups; GIII (11 strains), GV (1 strain), GVI (3 strains), GVII (6 strains), GVIII (1 strain), GX (3 strains), and GXI (4 strains). This manuscript presents the first nearly complete genome sequences of GX and GXI, and demonstrates novel intergenogroup recombination events.

1. Introduction

Sapoviruses (SaVs) are etiological agents of gastroenteritis in humans and animals and they have non-enveloped positive-sense, single-stranded RNA genome of approximately 7.1–7.7 kb in length with the typical morphology, the Star-of-David structure, as determined by electron microscopy (Oka et al., 2015; Saif et al., 1980). The SaV genome contains two overlapping open reading frames (ORFs) that encode the nonstructural proteins NS1-NS2-NS3 (putative NTPase)-NS4-NS5 (genome linked viral protein: VPg)-NS6 (Protease: Pro)-NS7 (RNA dependent RNA polymerase: RdRp) followed by the capsid protein, VP1, and the minor structural protein, VP2 (Oka et al., 2015).

Porcine SaV was first identified by electron microscopy in the United States of America in 1980 (Saif et al., 1980). SaVs have since been identified mostly using reverse transcriptase (RT)-PCR. The metagenomics approach, which does not require sequence-specific primers for PCR amplification, has recently allowed the detection of SaVs. SaVs have been reported from diarrheic and asymptomatic pigs (Cunha et al., 2010; das Merces Hernandez et al., 2014; Di Bartolo et al., 2014; Dufkova et al., 2013; Jeong et al., 2007; Keum et al., 2009; Kim et al., 2006; Liu et al., 2012; Liu et al., 2014; Martella et al., 2008; Martínez et al., 2006; Mauroy et al., 2008; Mijovski et al., 2010; Reuter et al., 2010; Scheuer et al., 2013; Valente et al., 2016; Wang et al., 2006; Zhang et al., 2014; Zhang et al., 2008). Cell culture-adapted and wild-

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Table 1
Summary of samples used in this study and a number of sapovirus contigs identified in each sample.

Sample name	Sample status	Region	Collection date	Days (months)	Health status	Number of sapovirus contigs	Sapovirus genogroup	Co-infection with other viruses
HkKa2-1/2015	Single	Tottori	2015.7.1	2 months	Without diarrhea	1	GV	Porcine astrovirus, Picobirnavirus, Posavirus
HgTa2-1/2015	Single	Tottori	2015.7.2	2 months	Without diarrhea	1	GVII	Porcine astrovirus, Porcine enterovirus, Sapelovirus, Porcine rotavirus C
HgTa2-2/2015	Single	Tottori	2015.7.2	2 months	Without diarrhea	2	GVII, GXI	Porcine astrovirus, Picobirnavirus
MoI2-1/2015	Single	Tottori	2015.7.2	2 months	Without diarrhea	2	GIII, GVII	Porcine astrovirus, Porcine enterovirus, Porcine picornavirus Japan
HgOg2-4/2015	Single	Tottori	2015.7.15	2 months	Without diarrhea	1	GVI	Porcine astrovirus, Porcine enterovirus, Porcine picornavirus Japan
Ishi-Im1/2015	Pooled	Ishikawa	2015.11.6	54	Without diarrhea	4	GIII, GVII (2), GVIII	Porcine astrovirus, Posavirus, Techovirus, Picobirnavirus
Ishi-Im3/2015	Pooled	Ishikawa	2015.11.6	30	Without diarrhea	2	GIII, GVII	Porcine astrovirus, Sapelovirus, Posavirus, Picobirnavirus
Ishi-Kah3/2015	Pooled	Ishikawa	2015.11.5	16	Mild diarrhea	1	GIII	Porcine enterovirus, Porcine kobuvirus
Ishi-Im7/2016	Single	Ishikawa	2016.11.25	11	Without diarrhea	4	GIII, GVI, GX, GXI	Teschovirus, Porcine enterovirus, Posavirus, Porcine rotavirus A
Ishi-Im9/2016	Single	Ishikawa	2016.11.25	11	Without diarrhea	1	GVI	Teschovirus, Porcine enterovirus, Picobirnavirus, Porcine kobuvirus, Porcine rotavirus A, Porcine rotavirus C
Ishi-Kah6/2016	Single	Ishikawa	2016.11.30	16	Without diarrhea	1	GIII	Porcine enterovirus, Porcine kobuvirus, Porcine rotavirus C
HgYa1/2016	Single	Tottori	2016.12.2	2 months	Without diarrhea	3	GIII, GXI (2)	Porcine astrovirus, Sapelovirus, Porcine enterovirus, Porcine rotavirus A, Porcine rotavirus C
HgYa2/2016	Single	Tottori	2016.12.2	2 months	Without diarrhea	2	GIII (2)	Porcine astrovirus, Sapelovirus, Porcine rotavirus B, Porcine rotavirus C
HgTa1/2016	Single	Tottori	2016.12.2	2 months	Without diarrhea	1	GIII	Porcine astrovirus, Picobirnavirus
HgTa2/2016	Single	Tottori	2016.12.2	2 months	Without diarrhea	1	GX	Porcine enterovirus, Picobirnavirus
HgTa3/2016	Single	Tottori	2016.12.2	2 months	Without diarrhea	2	GIII, GX	Porcine astrovirus, Porcine enterovirus, Porcine rotavirus B

type SaVs were shown to induce diarrhea in experimentally infected gnotobiotic piglets (Flynn et al., 1988; Guo et al., 2001; Lu et al., 2015). Therefore, SaVs are currently suggested as one of the etiological agents of gastroenteritis in pigs; however, the role of SaV as the cause of gastroenteritis in pigs has been obscure.

Initially, Farkas et al. classified human SaVs into GI-GV using entire VP1 sequences (Farkas et al., 2004). Thereafter, Scheuer et al. classified SaVs including those from humans and animals into 14 genogroups based on the complete VP1 sequences (Scheuer et al., 2013). Thus far, SaVs are divided into fifteen genogroups (Oka et al., 2016). To date, at least eight SaV genogroups: GIII, GV, GVI, GVII, GVIII, GIX, GX, and GXI, have been identified from pigs (Scheuer et al., 2013; Oka et al., 2016). In Japan, GIII, GV, GVII, GVIII, and GX genogroups of SaV have been found from finisher pigs and pigs, whose ages were < 5 months (Nakamura et al., 2010; Yin et al., 2006). Complete or nearly complete genome sequences of SaV GIII, GV, GVI, GVII, and GVIII are available in the DDBJ/EMBL/GenBank database, but those of GIX, X, and GXI are not available. In the present study, 26 nearly complete genome sequences and three partial sequences of SaV were obtained from fecal samples of pigs with or without diarrhea in Japan using a metagenomics approach. This study presents the first report of nearly complete genome data for SaV GX and GXI. Genetic analyses revealed high genetic diversity among SaVs in Japanese pig population and possible intergenogroup recombination events.

2. Materials and methods

2.1. Fecal samples, RNA extraction, and deep sequencing

In total, 105 fecal samples collected from 2 to 120-day-old pigs from 12 farms in Tottori Prefecture and Ishikawa Prefecture of Japan in 2015–2016 were used in this study. Samples were collected from pigs with diarrhea (six single samples and 17 pooled samples) or those without diarrhea (70 single samples and 12 pooled samples). Although pooled samples were impossible for recombination analysis, we accepted those samples for search for new genogroups only. Samples were diluted 1:9 (*w/v*) in sterile phosphate-buffered saline and centrifuged at $10{,}000 \times g$ for 10 min. The supernatants were collected and stored at -80 °C until required. Viral RNA was extracted from the fecal sample supernatants, using TRIzol® LS Reagent (Life Technologies, Carlsbad, CA, USA), and was treated with DNase I (Takara Bio, Shiga, Japan). cDNA libraries for deep sequencing were constructed using the NEBNext Ultra RNA Library Prep Kit for Illumina (New England Biolabs, Ipswich, MA, USA) as described previously (Nagai et al., 2015). Deep sequencing was conducted using the MiSeq Reagent Kit v2 (300-cycles) (Illumina, San Diego, CA, USA) on a MiSeq bench-top sequencer (Illumina). FASTQ-formatted sequence data files were generated using the MiSeq Reporter (Illumina). Trimmed paired-end sequence reads were assembled into contigs by *de novo* assembly with the strictest parameter setting (mismatch cost, 2; insertion cost, 3; deletion cost, 3; length function, 0.9; and similarity function, 0.9) in the CLC Genomics Workbench 7.5.5 (CLC bio, Aarhus, Denmark). The nucleotide sequences of SaV strains obtained in this study were deposited in the DDBJ/EMBL/GenBank databases under the accession numbers LC215874–LC215902.

2.2. Genome analysis

Nucleotide (nt) and amino acid (aa) sequences were aligned using ClustalW (Thompson et al., 1997). Phylogenetic trees were constructed using the 29 SaV strains in this study together with available SaV sequences from the DDBJ/EMBL/GenBank database following the maximum-likelihood method with the best-fit evolutionary models in MEGA 5.22 (Tamura et al., 2011) for rtREV + G + I (RdRp), rtREV + G + I + F (VP1 aa), GTR + G + I (VP1 nt), and rtREV + G + F (VP2). The reliability of the phylogenetic tree obtained for each gene

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