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Research paper

Analysis of complete genome sequences of G9P[19] rotavirus strains from human and piglet with diarrhea provides evidence for whole-genome interspecies transmission of nonreassorted porcine rotavirus

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ABSTRACT

Whole genomes of G9P[19] human (RVA/Human-wt/THA/CMH-S070-13/2013/G9P[19]) and porcine (RVA/Pig-wt/THA/CMP-015-12/2012/G9P[19]) rotaviruses concurrently detected in the same geographical area in northern Thailand were sequenced and analyzed for their genetic relationships using bioinformatic tools. The complete genome sequence of human rotavirus RVA/Human-wt/THA/CMH-S070-13/2013/G9P[19] was most closely related to those of porcine rotavirus RVA/Pig-wt/THA/CMP-015-12/2012/G9P[19] and to those of porcine-like human and porcine rotaviruses reference strains than to those of human rotavirus reference strains. The genotype constellation of G9P[19] detected in human and piglet were identical and displayed as the G9-P[19]-I5-R1-C1-M1-A8-N1-T1-E1-H1 genotypes with the nucleotide sequence identities of VP7, VP4, VP6, VP1, VP2, VP3, NSP1, NSP2, NSP3, NSP4, and NSP5 at 99.0%, 99.5%, 93.2%, 97.7%, 97.7%, 85.6%, 89.5%, 93.2%, 92.9%, 94.0%, and 98.1%, respectively. The findings indicate that human rotavirus strain RVA/Human-wt/THA/CMH-S070-13/2013/G9P[19] containing the genome segments of porcine genetic backbone is most likely a human rotavirus of porcine origin. Our data provide an evidence of interspecies transmission and whole-genome transmission of nonreassorted G9P[19] porcine RVA to human occurring in nature in northern Thailand.

1. Introduction

Rotavirus is a common pathogen of acute gastroenteritis in infants and young children and also in a wide variety of animal species (Kapikian et al., 2001). In many cases, genetic analysis of rotavirus genome has clearly demonstrated the genetic relatedness of gene segments of rotavirus strains isolated from different species. It is possible that interspecies transmission and genetic reassortment between different strains of rotaviruses may occur frequently in nature (Fujiwara and Nakagomi, 1997; Iizuka et al., 1994; Nakagomi et al., 1990). Close contact between humans and animals may facilitate interspecies transmission and genetic reassortment to occur during co-infection with different strains of rotaviruses and resulting in the generation of progeny viruses with novel or atypical genotypes (Palombo, 2002). The increased detections of rotavirus strains bearing unusual combinations of human and animal rotavirus genotypes are well documented (Bányai et al., 2009; Khamrin et al., 2006, 2009; Ianiro et al., 2014; Mukherjee et al., 2011; Maneekarn et al., 2006; Martinez et al., 2014; Steyer et al., 2008). Thus far, genetic reassortment between group A rotaviruses (RVA) of human and animal origins has been reported, including human-porcine (Bányai et al., 2009; Ghosh et al., 2012; Maneekarn et al., 2006; My et al., 2014; Than et al., 2013), human-simian (Khamrin et al., 2006), human-bovine (Cooney et al., 2001; Martinez et al., 2014), human-feline/canine (Isegawa et al., 1992; Luchs et al., 2012), human-caprine (Khamrin et al., 2006), human-lapine (Matthijnssens et al., 2006), human-ovine (Bányai et al., 2010), and human-equine rotaviruses (Arana et al., 2016; Malasao et al., 2015).

The viral genome of RVA consists of 11 segments of double-stranded RNA (dsRNA) encoding for six structural proteins (VP1-VP4, VP6, and VP7) and five or six nonstructural proteins (NSP1-5/6) (Estes and Greenberg, 2013). The two outer capsid proteins, VP4 and VP7, have

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been used for classification of RVA into P and G genotypes, respectively. To date, at least 38 P genotypes and 27 G genotypes of RVA have been reported from humans and wide variety of animal species (Fujii et al., 2016; Matthijnssens et al., 2011; Trojnar et al., 2013). Most recently, RVA has been classified based on the assignment of genotypes to all 11 genome segments. The notation of Gx-P[x]-Ix-Rx-Cx-Mx-Ax-Nx-Tx-Ex-Hx, representing the genotypes of VP7-VP4-VP6-VP1-VP2-VP3-NSP1-NSP2-NSP3-NSP4 and NSP5, respectively, has been assigned by the Rotavirus Classification Working Group (RCWG) (Matthijnssens et al., 2011).

P[19] was first identified in pig in China (G3P[19]) (Burke et al., 1994) while the first human RVA P[19] strains was detected in Thailand (G9P[19]) (Okada et al., 2000). Later, only a few human RVA P[19] strains have been reported in combination with G1, G3, G5, and G9 genotypes from India (Krishnan et al., 1994; Mukherjee et al., 2011; Varghese et al., 2004; Zade et al., 2009), Taiwan (Wu et al., 2011), Vietnam (Nguyen et al., 2008), Italy (Ianiro et al., 2014). In pigs, P[19] was also detected in combination with G3, G4, G9, and G26 in Thailand (Maneekarn et al., 2006; Saikruang et al., 2013), Vietnam (My et al., 2014). In Thailand, unusual strains G9P[19], RVA/Human-wt/THA/Mc323/1989/G9P[19] and RVA/Human-wt/THA/Mc345/1989/G9P[19], so-called Mc323 and Mc345 strains, were initially isolated from children hospitalized with diarrhea in Chiang Mai province in 1989, and had been shown by RNA-RNA hybridization, nucleotide and amino acid sequence analyses of VP7 and VP4 genes to be more closely related to the porcine RVA than to human RVA (Okada et al., 2000; Urasawa et al., 1992). Most recently, whole genome sequences of Mc323 and Mc345 have been analyzed and found that most of the genes appeared to be of porcine origin (Ghosh et al., 2012). During the surveillance of RVA in pigs in Chiang Mai in 2000–2001, the G3P[19] porcine RVA strains were detected and their VP4 genes were most closely related to those of Mc323 and Mc345 (Maneekarn et al., 2006). However, the G9P[19] was not detected in that surveillance.

In the present study, whole genome sequences of G9P[19] concurrently detected in pediatric patient and in piglet with diarrhea during the surveillance of RVA in Chiang Mai in 2012–2013 were analyzed for their genetic relationships.

2. Materials and methods

2.1. Specimen collection

A total of 401 fecal specimens were collected from children admitted to the hospitals with acute gastroenteritis in Chiang Mai, Thailand during 2013–2014. In addition, 491 fecal specimens were collected from diarrheic piglets in Chiang Mai and in Lumphun provinces, Northern Thailand during 2011–2014. The presence of RVA in fecal specimens were screened and then identified for their G and P genotypes by multiplex-PCR method using genotype-specific primers (Gentsch et al., 1992; Gouvea et al., 1990; Gouvea et al., 1994; Winiarczyk et al., 2002). Among these, 137 (34.2%) were positive for human RVA and 113 (23.0%) were positive for porcine RVA.

Human RVA G9P[19] (1 out of 401) strain RVA/Human-wt/THA/CMH-S070-13/2013/G9P[19] was isolated from stool sample of a 3-year-old girl with diarrhea in January 2013 while porcine RVA G9P[19] (2 out of 491) strains RVA/Pig-wt/THA/CMP-013-12/2012/G9P[19] and RVA/Pig-wt/THA/CMP-015-12/2012/G9P[19] were isolated from stool samples of diarrheic piglets in May 2012 in Chiang Mai, Thailand. These two strains of porcine RVA G9P[19] were reported epidemiologically in the previous study (Yodmeeklin et al., 2016) and the complete genome sequence of one strain (CMP-015-12) was analyzed in the present study. The study was conducted with the approval of the ethical committee for human rights related to human experimentation, Faculty of Medicine, Chiang Mai University (MIC-2557-02710). The stool specimens from both human and piglet were stored at −20 °C until used.

2.2. Viral RNA extraction and complete genome nucleotide sequencing

The rotavirus dsRNA was extracted from the supernatant of 10% stool suspension in phosphate buffered saline (PBS) pH 7.4 using Geneaid Viral Nucleic Acid Extraction Kit II (Geneaid, Taipei, Taiwan), according to the manufacturer's protocol. The viral RNA was reverse transcribed using random primers and reverse transcriptase enzyme according to the manufacturer's instruction (Thermo Scientific, USA). The full-length nucleotide sequences of all 11 gene segments of human CMH-S070-13 and porcine CMP-015-12 were determined using specific primers for individual genes. The primers used for the amplification of different gene segments of these strains are listed in Table 1.

2.3. Sequence analysis and construction of phylogenetic tree

For RVA identification and genotyping, all amplified PCR products of each genome segments were purified using Gel/PCR DNA Fragments Extraction Kit (Geneaid, Taipei, Taiwan) according to the manufacturer's protocol. Then, the purified PCR products were sequenced by fluorescence based cycle sequencing method using BigDye® Terminator v3.1 Cycle Sequencing Reaction kit (Applied Biosystems, Foster city, CA, USA). The obtained nucleotide sequences of all 11 gene segments of G9P[19] human and porcine RVA strains were manually assembled and analyzed by using ClustalX (Thompson et al., 1997) and BioEdit (Hall, 1999) softwares. The obtained sequences were used to search for a close genetic relationship with the reference sequences using BLAST server (http://blast.ncbi.nlm.nih.gov/Blast.cgi) in NCBI database. Those reference sequences obtained from BLAST search and the sequences of RVA strains CMH-S070-13 and CMP-015-12 were aligned using ClustalX (1.81) software. Phylogenetic analysis was performed with MEGA software version 7.0 based on the neighbor-joining method (Kumar et al., 2016). The tree was statistically supported by bootstrapping with 1000 replicates, and phylogenetic distances were calculated using the Kimura 2-parameter model.

2.4. Nucleotide sequence accession numbers

Nucleotide sequences of human and porcine RVA strains described in the present study have been deposited in the GenBank under the accession numbers KU363137-KU363138 (VP1), KU363151-KU363152 (VP2), KU363147-KU363148 (VP3), KU363153-KU363154 (VP4), KU363133-KU363134 (VP6), KU363149-KU363150 (VP7), KU363145-KU363146 (NSP1), KU363135-KU363136 (NSP2), KU363139-KU363140 (NSP3), KU363141-KU363142 (NSP4), and KU363143-KU363144 (NSP5) for CMH-S070-13 and CMP-015-12 strains, respectively.

3. Results

3.1. Genotype constellation

The complete genotype constellation of a human RVA strain RVA/Human-wt/THA/CMH-S070-13/2013/G9P[19] and a porcine RVA strain RVA/Pig-wt/THA/CMP-015-12/2012/G9P[19] detected in the present study were identical and both were assigned as G9-P[19]-I5-R1-C1-M1-A8-N1-T1-E1-H1 genotypes for the VP7-VP4-VP6-VP1-VP2-VP3-NSP1-NSP2-NSP3-NSP4-NSP5/6 genes, respectively. Comparisons of the complete genotype constellations of strains CMH-S070-13 and CMP-015-12 with those of other G9P[19] and non-G9P[19] RVA reference strains are shown in Table 2. The complete genotype constellation of CMH-S070-13, a human RVA, was also identical with those of porcine-like human RVA strain G9P[19] (Mc323 and Mc345) and human RVA (mani-97). Additionally, the genotype constellation of CMH-S070-13 was also identical with those of other porcine-like human RVA including G9P[6] (BE2001), G26P[19] (30378), and G5P[6] (Ryukyu-1120).