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Genomic characterization of G3P[6], G4P[6] and G4P[8] human rotaviruses from Wuhan, China: Evidence for interspecies transmission and reassortment events

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ABSTRACT

We report here the whole genomic analyses of two G4P[6] (RVA/Human-wt/CHN/E931/2008/G4P[6], RVA/Human-wt/CHN/R1954/2013/G4P[6]), one G3P[6] (RVA/Human-wt/CHN/R946/2006/G3P[6]) and one G4P[8] (RVA/Human-wt/CHN/E2484/2011/G4P[8]) group A rotavirus (RVA) strains detected in sporadic cases of diarrhea in humans in the city of Wuhan, China. All the four strains displayed a Wa-like genotype constellation. Strains E931 and R1954 shared a G4-P[6]-I1-R1-C1-M1-A8-N1-T1-E1-H1 constellation, whilst the 11 gene segments of strains R946 and E2484 were assigned to G3-P[6]-I1-R1-C1-M1-A1-N1-T1-E1-H1 and G4-P[8]-I1-R1-C1-M1-A1-N1-T1-E1-H1 genotypes, respectively. Phylogenetically, the VP7 gene of R946, NSP3 gene of E931, and 10 of 11 gene segments of E2484 (except for VP7 gene) belonged to lineages of human RVAs. On the other hand, based on available data, it was difficult to ascertain porcine or human origin of VP3 genes of strains E931 and R946, and NSP2 genes of strains R946 and R1954. The remaining genes of E2484, E931, R946 and R1954 were close to those of porcine RVAs from China, and/or porcine-like human RVAs. Taken together, our observations suggested that strain R1954 might have been derived from porcine RVAs, whilst strains R946 and E931 might be reassortants possessing human RVA-like gene segments on a porcine RVA genetic backbone. Strain E2484 might be derived from reassortment events involving acquisition of a porcine-like VP7 gene by a Wa-like human RVA strain. The present study provided important insights into zoonotic transmission and complex reassortment events involving human and porcine RVAs, reiterating the significance of whole-genomic analysis of RVA strains.

1. Introduction

Group A rotavirus (RVA), a member of the family Reoviridae, is the leading cause of severe diarrhea in the young of humans, animals and birds (Estes and Kapikian, 2007). The RVA genome consists of 11 segments of double-stranded RNA that encode six structural proteins (VP1–VP4, VP6 and VP7) and six nonstructural proteins (NSP1–NSP6) (Estes and Kapikian, 2007). Based on the differences in nucleotide sequences of the RVA VP7 and VP4 genes, to date, RVA strains have been classified into at least 27 G and 37 P genotypes, respectively (Estes and Kapikian, 2007; Matthijnssens et al., 2011; Trojnar et al., 2013). Among them, G1, G2, G3, G4, G9, and G12 in combination with P[4], P[6], and P[8] are frequently detected in human RVAs throughout the world (Gentsch et al., 1996; Santos and Hoshino, 2005; Matthijnssens et al., 2010). On the other hand, porcine RVAs have been found to commonly possess G3, G4, G5, G9, and G11 in conjunction with P[6] and P[7] (Papp et al., 2013a). Genetic studies on rotaviruses, especially whole genome analysis, have provided evidence for close relationships between human and porcine RVAs. The major human genotype constellation, Wa genogroup, was described as having a

Abbreviation: group A rotavirus, RVA.

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common origin with porcine rotaviruses (Matthijnssens et al., 2008). Therefore, studies on interspecies transmission and reassortment events involving porcine and human RVA strains are crucial to further deciphering the complex evolutionary patterns and ecology of human RVAs.

The evolution and genetic diversity of rotaviruses are governed by point mutations, reassortment, rearrangement and intragenic recombination (Estes and Kapikian, 2007). Although host species barriers and host range restriction exist in rotavirus, interspecies transmission also contributes to the diversity and evolution of rotavirus, and is usually accompanied by reassortment. It has been recognized that porcine rotaviruses might be able to infect human by interspecies transmission. The most prevalent VP7 and VP4 genotypes among porcine RVAs are G5 and P[7], followed by G4 and P[6] (Papp et al., 2013a). However, in humans, G4P[6] RVAs, possibly derived from pigs, are more frequent than G5P[7]. Most of porcine-like human P[6] strains have been reported in combination with G4, followed by G5, G9, and G3 (Bányai et al., 2009; Degiuseppe et al., 2013; Do et al., 2014; Dong et al., 2013; Duan et al., 2007; Hwang et al., 2012; Komoto et al., 2013; Martella et al., 2008; Martinez et al., 2014; Mladenova et al., 2012; Mukherjee et al., 2009, 2011; Nguyen et al., 2007; Papp et al., 2013b; Wang et al., 2010; Zeller et al., 2012).

The complete genomes of at least 23 porcine-like human RVA strains and 27 porcine RVA strains have been published so far (Ghosh et al., 2010, 2012a; Kim et al., 2012; Martel-Paradis et al., 2013; Martinez et al., 2014; Matthijnssens et al., 2008; Monini et al., 2014; Mullick et al., 2013; Okitsu et al., 2013; Theuns et al., 2015). Of these, only 2 G9P[23] porcine RVAs, 5 G4P[6] and 1 G5P[6] porcine-like human RVAs were detected in China (Dong et al., 2013; Duan et al., 2007; Shi et al., 2012; Wang et al., 2010). Among these G4P[6] RVAs, four strains (GX54, GX77, GX78, and GX82) from Guangxi region shared a G4-P[6]-I1-R1-C1-M1-A8-N1-T1-E1-H1 genotype constellation, with almost 100% nucleotide identities in all the 11 genomic cognate gene segments (Dong et al., 2013). By whole genomic analysis, the remaining G4P[6] strain, R479, detected in Wuhan city in 2004, appeared to be of porcine origin (Wang et al., 2010). In the present study, the nearly full-length nucleotide sequences of all the 11 gene segments of two recent G4P[6] (2008 and 2013), one G3P[6] and one G4P[8] human RVA strains from Wuhan were determined and analyzed together with those of contemporary common HRVs from Wuhan (Shintani et al., 2012; Wang et al., 2011, 2014), and porcine-like human and porcine RVAs around the world to understand the genetic relationships between human and porcine rotaviruses.

2. Materials and methods

2.1. Virus strains

Strains RVA/Human-wt8/CHN/E931/2008/G4P[6] (E931), RVA/Human-wt/CHN/R1954/2013/G4P[6] (R1954), RVA/Human-wt/CHN/R946/2006/G3P[6] (R946), and RVA/Human-wt/CHN/E2484/2011/G4P[8] (E2484) were detected in diarrheal stool samples collected from a 3-month-old male, an 8-month-old female, and two 1-year-old male infants in the city of Wuhan, Hubei province, China, from 2006 through 2013, respectively.

2.2. Nucleotide sequencing, genotyping and sequence analyses

The nucleotide sequence of the VP7 gene of strain E931 was obtained previously (Wang et al., 2009). Therefore, the nucleotide sequences of the remaining genes of E931 and whole genome of strains R946, R1954 and E2484 were determined in this study. Viral RNA was extracted from stool samples using the QIAamp Viral RNA Mini Kit (Qiagen GmbH, Germany). RT-PCRs were performed using the QIAGEN One Step RT-PCR Kit (Qiagen GmbH, Germany). Primers used for the amplification of different RVA genes are shown in Supplementary Table S1. Nucleotide sequences were determined using the BigDye Terminator v3.1 Cycle Sequencing kit (Applied Biosystems, CA, USA) on an automated DNA sequencer (ABI PRISM 3730).

Genotype of each segment was assigned by Rota C 2.0, an online automated RVA genotyping tool (http://rotac.regatools.be/) (Maes et al., 2009). The sequence similarities were analyzed by using BLAST (http://blast.ncbi.nlm.nih.gov/Blast.cgi). Multiple alignments of the determined sequences were performed using MAFFT (http://mafft.cbrc.jp/alignment/software/) program. Phylogenetic trees of individual segments were constructed by Maximum Likelihood (ML) method using MEGA (v5.05) software (Tamura et al., 2011). The representative strains of porcine-like human and porcine rotaviruses collected from different regions during different periods were included in the phylogenetic trees. The Hasegawa–Kishino–Yano model was used in ML method to construct the phylogenetic trees for all gene segments, and the trees were statistically supported by bootstrapping with 1000 replicates. Other models, such as the Tamura 3-parameter model, Tamura–Nei model, Jukes–Cantor model, General Time Reversible model and Kimura 2-parameter model, were used to corroborate the results of phylogenetic analysis. The clustering patterns of our study strains were found to be same, which rules out any biases among models with regards to the study strains. Putative lineages and sublineages within a genotype (Fig. 1A–K) were assigned arbitrarily based on observation of clustering patterns. We also followed the classification of lineages described in previously published literatures (Dong et al., 2013; Lorenzetti et al., 2011; Martella et al., 2008; Martinez et al., 2014; Mladenova et al., 2012; Mukherjee et al., 2009; Wang et al., 2010). The bootstrap values of the lineages in these phylogenetic trees were not less than 80. Nucleotide identities corroborated the results of phylogenetic analysis.

2.3. Nucleotide sequence accession numbers

The GenBank accession numbers for the nucleotide sequences of the genomes of strains E931, E2484, R946 and R1954 are EU708602, KF726034-KF726076.

3. Results

3.1. Genotype constellation

Whole genome-based genotypes of E931 and R1954 were both assigned as G4-P[6]-I1-R1-C1-M1-A8-N1-T1-E1-H1. The genotypes of R946 and E2484 were G3-P[6]-I1-R1-C1-M1-A1-N1-T1-E1-H1 and G4-P[8]-I1-R1-C1-M1-A1-N1-T1-E1-H1, respectively (Tables 1 and 2). All the four strains displayed a Wa-like genotype constellation. Among these strains, nucleotide identities of G4-VP7 genes and P[6]-VP4 genes were 95–96% and 94–96%, respectively. Among the P[6] strains, nucleotide identities of the VP1, VP2, VP4, VP6, and NSP5 genes were more than 95%, whereas lower identities were observed in the VP3, NSP1, NSP2, and NSP3 genes (86%, 80%, 90%, and 89%, respectively).

3.2. Sequence analyses of structural protein genes

Phylogenetic tree of the VP7 genes (Fig. 1A) indicated that E931, R1954, and E2484 were grouped into lineage G4h of G4 rotaviruses, which included porcine rotaviruses HeN4 and HLJhg2 from China, CMP77 from Thailand and FGP36 from Japan, and some porcine-like human rotaviruses from China, Vietnam, India, Russia,

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