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Research paper

Whole genomic analysis of human and bovine G8P[1] rotavirus strains isolated in Nigeria provides evidence for direct bovine-to-human interspecies transmission

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ABSTRACT

Bovine group A rotavirus (RVA) G8P[1] strains have been rarely detected in humans. Two Nigerian G8P[1] strains, HMG035 (RVA/Human-tc/NGA/HMG035/1999/G8P[1]) and NGRBg8 (RVA/Cow-tc/NGA/NGRBg8/1998/G8P[1]), were previously suggested to have the VP7, VP4, and NSP1 genes of bovine origin. In order to obtain precise information on the origin and evolution of these G8P[1] strains, the complete nucleotide sequences of the whole genomes of strains HMG035 and NGRBg8 were determined and analyzed in the present study. On whole genomic analysis, strains HMG035 and NGRBg8 were found to be very closely related to each other in all the 11 segments, and were found to have a bovine RVA-like genotype constellation (G8-P[1]-I2-R2-C2-M2-A11-N2-T6-E2-H3). Furthermore, on phylogenetic analysis, each of the 11 genes of strains HMG035 and NGRBg8 appeared to be of bovine origin. Thus, strains HMG035 and NGRBg8 were suggested to be derived from a common origin, and strain NGRBg8 was assumed to represent an example of bovine RVA strains that were transmitted to humans. Our findings provide clear evidence for direct bovine-to-human interspecies transmission of RVA strains.

1. Introduction

Group A rotaviruses (RVAs), members of the *Reoviridae* family, are the leading cause of severe gastroenteritis in the young of humans and many animal species worldwide. In humans, RVA infections are associated with high morbidity and mortality, accounting for an estimated 453,000 deaths among children <5 years of age annually (Tate et al., 2012). The mature RVA virion is a triple-layered, non-enveloped icosahedron that encapsidates an 11-segment genome of double-stranded (ds)RNA (Estes and Greenberg, 2013).

RVAs have two outer capsid proteins, VP7 and VP4, which are antigenetically significant as they are implicated independently in neutralization, and define the G and P genotypes, respectively. To date, RVAs have been classified into 27 G and 37 P genotypes (Matthijnssens et al., 2011; Trojnar et al., 2013). Among them, some specific G and P genotypes are dominant in individual host species (Martella et al., 2010; Tacharoenmuang et al., 2015). In human RVAs, G genotypes G1–4, G9, and G12, and P genotypes P[4], P[6], and P[8] are considered as major genotypes (Martella et al., 2010; Santos and Hoshino, 2005). In addition, several G genotypes (G5, G6, G8, G10, G11, and G20) and P genotypes (P[1]–[3], P[5], P[7], P[9]–[11], P[14], P[19], and P[25]) have been detected sporadically in the human population (E1 Sherif et al., 2011; Gentsch et al., 2005; Solberg et al., 2009; Tacharoenmuang et al., 2015). Many of these unusual genotypes are believed to have originated from animal RVA strains that were introduced into humans through interspecies transmission events, one of the major ways through which the genetic diversity of RVAs has been generated (Ghosh and Kobayashi, 2011; Komoto et al., 2013, 2014a; Martella et al., 2010; Papp et al., 2013).

G8, which like G6 and G10 is one of the most common G genotypes in cattle (Jere et al., 2012; Martella et al., 2010; Papp et al., 2013), was first detected in cattle in 1965 (Small et al., 2007). G8 strains have also been identified in humans in combination with either of a P[1], P[4], P[6], P[8], P[10], or P[14] genotype (Ghosh et al., 2011; Hoshino et al., 2006; Matthijnssens et al., 2011). P[1] strains, the P genotype commonly found in artiodactyls such as cattle, have also been found in children in combination with a G8 genotype (Ghosh et al., 2011; Matthijnssens et al., 2011). The first human G8P[1] strain, B12, was identified in an asymptomatic child in Kenya in 1987 (Ghosh et al., 2011), and subsequently in diarrheic children in Ghana (Heylen et al., 2015), India (Jagannath et al., 2000), Nicaragua (Bányai et al., 2009), Nigeria (Adah et al., 2001), and Paraguay (Martinez et al., 2014). In host species other than humans, G8P[1] strains have been identified in artiodactyls (cattle, goats, and guanacos) (Adah et al., 2003; Chang et al., 1995; Jere et al., 2012; Louge Uriarte et al., 2014; Matthijnssens et al., 2009;

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Parreño et al., 2004; Small et al., 2007; Taniguchi et al., 1991), dogs (Sieg et al., 2015), horses (Isa et al., 1996), and monkeys (Hoshino et al., 2006). We detected two G8P[1] strains, HMG035 (RVA/Human-tc/NGA/HMG035/1999/G8P[1]) and NGRBg8 (RVA/Cow-tc/NGA/NGRBg8/1998/G8P[1]), in two diarrheic fecal specimens from a child and a calf, respectively, in near geographic regions in Nigeria in 1998–1999 (Adah et al., 2001, 2003).

A whole genome-based genotyping system was recently proposed for RVAs with the aim of facilitating tracing of the origins and evolutionary dynamics of RVAs (Matthijnssens et al., 2008a). Whole genome-based analysis is a reliable method for obtaining conclusive data on the origin of an RVA strain, and for tracing its evolutionary dynamics (Ghosh and Kobayashi, 2011; Komoto et al., 2015; Matthijnssens et al., 2008a, 2011). To date, however, the whole genomes of only several G8P[1] strains from Africa and the Americas have been analyzed, which provided evidence of their artiodactyl (likely bovine) origin (Bányai et al., 2009; Ghosh et al., 2011; Heylen et al., 2015; Hoshino et al., 2006; Jere et al., 2011; Matthijnssens et al., 2009; Small et al., 2007). G8P[1] strains are speculated to be the result of reassortment between strains from cattle and other member(s) of the Artiodactyla (Ghosh et al., 2011; Jere et al., 2012). Nucleotide sequencing of partial genomes (VP7, VP4, and NSP1 genes) and RNA-RNA hybridization studies suggested a bovine origin of Nigerian G8P[1] strains HMG035 and NGRBg8 (Adah et al., 2001, 2003). Partial genomic analyses of other G8P[1] strains from Central America, Asia, and Europe have also shown the presence of bovine-like gene segments (Jagannath et al., 2000; Martinez et al., 2014; Sieg et al., 2015; Taniguchi et al., 1991). However, the overall genomic constellation and exact evolutionary patterns of G8P[1] strains remain to be elucidated. Therefore, the complete nucleotide sequences of the whole genomes of strains HMG035 and NGRBg8 were determined and analyzed in the present study.

2. Materials and methods

2.1. Virus strains

The full-genomic sequences were determined for strains HMG035 and NGRBg8, which were identified in two diarrheic fecal specimens from a child and a calf, respectively, during the RVA surveillance program in Nigeria in 1998–2000, which involved a total of 235 (127 human and 108 bovine) fecal specimens (Adah et al., 2001, 2003). The sampling sites for strains HMG035 and NGRBg8 were close (Adah et al., 2003). Strains HMG035 and NGRBg8 were subsequently adapted to MA104 cells (Adah et al., 2001, 2003).

2.2. Viral dsRNA extraction

Viral dsRNAs of strains HMG035 and NGRBg8 were extracted from the cell cultures using a QIAamp Viral RNA Mini Kit (Qiagen). The extracted dsRNAs were used for (i) polyacrylamide gel electrophoresis (PAGE) analysis, and (ii) whole genomic analysis. For PAGE analysis, the dsRNAs were electrophoresed in a 10% polyacrylamide gel for 16 h at 20 mA at room temperature, followed by silver staining (Komoto et al., 2006) to confirm the genomic dsRNA profiles. For whole genomic analysis, viral dsRNAs were subjected to Illumina MiSeq sequencing as described below.

2.3. cDNA library building, Illumina MiSeq sequencing, and bioinformatics analysis

Preparation of a cDNA library and Illumina MiSeq sequencing were performed as described previously (Ide et al., 2015; Komoto et al., 2014a, 2015; Tacharoenmuang et al., 2015). Briefly, a 200 bp fragment library ligated with bar-coded adapters was constructed for strains HMG035 and NGRBg8 using an NEBNext Ultra RNA Library Prep Kit for Illumina v1.2 (New England Biolabs) according to the manufacturer's instructions. Library purification was performed using Agencourt AMPure XP magnetic beads (Beckman Coulter). Nucleotide sequencing was performed on an Illumina MiSeq sequencer (Illumina) using a MiSeq Reagent Kit v2 (Illumina) to generate 151 paired-end reads. The raw data were base called and reads with the same barcode were collected and assigned to a sample on the instrument, which generated Illumina FASTQ files. Data analysis was carried out using CLC Genomics Workbench v8.0.1 (CLC Bio). We first trimmed the raw data by removing low quality and short reads, followed by merging all the paired-end reads into single reads. Second, bar-coded adapters were removed from the reads. Third, all ambiguous (N) bases were trimmed from the reads. Forth, contigs were assembled from the remaining sequence reads by *de novo* assembly. We removed the contigs with a contig length below 500. Finally, using the assembled contigs as query sequences, the Basic Local Alignment Search Tool (BLAST) non-redundant nucleotide database was searched to obtain the full-length nucleotide sequence of each gene segment of strains HMG035 and NGRBg8. To further improve the contigs of strains HMG035 and NGRBg8, the sequence reads of each segment were mapped back to the assembled contigs of these two strains.

2.4. Determination of RNA genotypes

The genotype of each of the 11 gene segments of strains HMG035 and NGRBg8 was determined using the RotaC v2.0 automated genotyping tool (http://rotac.regatools.be/) (Maes et al., 2009) according to the guidelines proposed by the Rotavirus Classification Working Group.

2.5. Phylogenetic analyses

Sequence comparisons were performed as described previously (Ide et al., 2015; Komoto et al., 2014a, 2015; Tacharoenmuang et al., 2015). Briefly, multiple alignment of each viral gene was performed using CLUSTAL W. Pairwise sequence identities were calculated using GENETYX v11 (GENETYX). Phylogenetic trees were constructed using the maximum likelihood method and the Tamura-Nei substitution model using MEGA6.06 (Tamura et al., 2013). The reliability of the branching order was estimated from 1000 bootstrap replicates (Felsenstein, 1985). The results of phylogenetic analyses were validated using several other genetic distance models, such as the Kimura 2-parameter, Tamura 3-parameter, Hasegawa-Kishino-Yano, and Jukes-Cantor ones, ruling out any biases among different models concerning the study strains (data not shown).

2.6. Nucleotide sequence accession numbers

The nucleotide sequence data presented in this paper have been deposited in the DDBJ and EMBL/GenBank data libraries. The accession numbers for the nucleotide sequences of the VP1-4, VP6, VP7, and NSP1-5 genes of strains HMG035 and NGRBg8 are LC119093-LC119103 and LC119104-LC119114, respectively.

3. Results and discussion

3.1. Profiles of genomic dsRNAs of strains HMG035 and NGRBg8 on PAGE

The virion dsRNAs of strains HMG035 and NGRBg8 were extracted from the cell cultures and then analyzed by PAGE. Supplementary Fig. S1 shows the profiles on PAGE of viral dsRNAs from strains HMG035 (lane 2) and NGRBg8 (lane 3). Both strains had a long electropherotype, as reported for most bovine strains (Gerna et al., 1990). Of note was that strains HMG035 and NGRBg8 had an almost identical electropherotype, the segment 5 (NSP1) genes differing, suggesting a close genetic relatedness between the two strains. The NSP1 gene of strain HMG035 was found to possess a long nucleotide deletion (219 bp) within the NSP1