Complete Genome Sequencing of a Recombinant Strain between HAstV-2 and HAstV-8 Isolated in South Korea

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Abstract

Human astroviruses (HAstVs) occur worldwide and are known to the causative agents of diarrhea in infants and elderly patients with immune dysfunction. This study aimed to identify recombinant HAstV strains and characterize rare genotypes. The full-length genome of a recombinant HAstV strain isolated from the stool sample of a patient with acute gastroenteritis from South Korea was amplified using three pairs of previously designed primers and seven newly designed primers. The recombinant HAstV was 6,757-bp long and contained three sequential open reading frames (ORFs), designated as ORF1a (2,781 bp), ORF1b (1,548 bp), and ORF2 (2,349 bp). Our findings suggested that a recombination event had occurred between ORF1b and ORF2 of the isolated strain, with a recombination breakpoint at 4,081 bp. To our knowledge, this is the first study to reveal the complete nucleotide sequence of a recombinant HAstV strain from South Korea. Our study findings might be useful for identifying other recombinant HAstV strains and for developing vaccines against this pathogenic virus.

Keywords: Astrovirus, Recombinant strain; Complete genome sequencing

Introduction

Human astroviruses (HAstVs; genus, Mamastrovirus; family, Astroviridae) are one of the major causes of acute gastroenteritis in infants worldwide [1] and acute diarrhea in young children [2]; the infection is more prevalent from autumn to winter. About 2–8% cases of diarrhea in children are caused by HAstV [3]. This virus was first discovered in 1975 by electron microscopy observation following an outbreak of diarrhea in humans [4]. Humans infected with HAstV show symptoms of body trembling, diarrhea, headache, vomiting, abdominal pain, and fever. HAstV infection is transmitted via the fecal-oral route, person-to-person contact, or contaminated food or water. The incubation period for this virus is 3–4 days after infection [5].

HAstVs have a single-stranded positive-sense RNA genome with a non-enveloped icosahedral capsid. The entire genome is about 6.8 kb [6] and contains three overlapping open reading frames (ORFs). ORF1a encodes an RNA-dependent polymerase; ORF1b, an RNA-dependent RNA polymerase; and ORF2, a capsid precursor protein [7]. The capsid protein can be divided into the N-terminal domain, a hypervariable domain, and a highly acidic C-terminal domain [8,9]. Thus far, eight types of HAstVs have been identified [10]. HAstV-1 is the most frequent, and the other genotypes are rare [11-13].

In Italy, the differences in ORF regions of HAstV isolates belonging to different genetic lineages were investigated [14]. In China, HAstV-1 infections are the most frequent and mostly occur in winter [15]. This virus is known to undergo genetic recombination and is thus difficult to control. Between 2002 and 2007 HAstV infections in South Korea were mainly caused by HAstV-1 [16,17]. HAstV, worldwide frequently occur disease, occurs is a rare serotype is looking for specificity by onset is recombination, were studied. In this study, the characteristics of a HAstV strain that had undergone genetic recombination were determined. This strain was isolated from the stool sample of a patient having acute gastroenteritis from South Korea. In addition, we performed complete genome sequencing of the newly detected recombinant strain.

Materials and Methods

Stool sample preparation

The HAstV-positive stool sample was obtained from a male infant patient with acute enteritis in March 2014 from WAVA in South Korea. The stool sample was stored at -70°C until RNA extraction.

Viral RNA extraction

The frozen stool sample was prepared as a 10% suspension in phosphate-buffered saline (pH 7.2) and centrifuged for 30 min at 13,000 rpm at 4°C. Viral RNA was extracted from 140 μL of the supernatant using a QIAamp Viral RNA Mini Kit (Qiagen, Hilden, Germany), following the manufacturer’s protocol. The extracted RNA was used as a template for reverse transcription-polymerase chain reaction (RT-PCR).

RT-PCR analysis

For the detection of HAstV, RT-PCR was performed using the One-Step RT-PCR Kit (Qiagen, Hilden, Germany) by using Mon269 and Mon 270 primers developed based on the sequence of the HAstV ORF2 region (Table 1). For sequencing the entire genome of the detected HAstV strain, RT-PCR was performed using the OneStep RT PCR Kit and an S1000TM Thermal Cycler (Bio-Rad, Hercules, CA, USA) by using 10 sets of primers—three pairs of previously designed primers and

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seven newly designed primers (Table 1). The new primers were designed based on the published sequences of type 8 variants of HAstV. In all, 10 fragments were amplified: 4 fragments for ORF1a, 2, for ORF1b; and 4, for ORF2. The conditions for RT-PCR were as follows: reverse transcription performed using the 3'-end poly (A) tail-based 3'-oligo (dT)-anchor primer (Table 1) under the following conditions: 30 cycles of 98°C for 10 s, 55°C for 30 s, and 72°C for 1 min, followed by extension at 72°C for 7 min, according to the manufacturer's instructions. The PCR products were run on 2% agarose gels containing ethidium bromide.

**Determination of the 5'- and 3'-ends of the HAstV genomic RNA**

The complete sequence of the 5'-end of the HAstV genomic RNA was determined by rapid-amplification of cDNA ends RACE by using the 5'-Full RACE Core Set Kit (TaKaRa Bio Inc., Ohtsu, Japan) and an S1000™ Cycler. The first cDNA strand was synthesized via reverse transcription from the target mRNA by using 5' ʹ-end-phosphorylated RT primers (Table 1); subsequently, the cDNA strand was treated with RNase H (for hybrid RNA removal) and RNA ligase-based 3' Oligo (dT)-anchor CAA TGA GGT TAT GGC TTT GGA ACT TTT TTT - (Table 1). Multiple sequence alignments were performed using Clustal W version 6.06). Phylogenetic trees were created using the neighbor-joining method by using Molecular Evolutionary Genetic Analysis software (MEGA version 6.06). Phylogenetic trees were created using the neighbor-
SimPlot analysis

The full sequence of the isolated strain was analyzed using the SimPlot ver.4 program to determine the breakpoint of recombination by using window and step sizes of 200 bp and 20 bp, respectively.

Results

HAstV detection

In this study, we analyzed the nucleotide sequence of the strain (Kor85) isolated from South Korea. The 6,757-nucleotide long sequence included 82-bp long 5'UTR and 69-bp long 3'UTR. The whole genome sequence analysis revealed 3 overlapping ORFs: ORF1a, 83–2863 (2,781 bp); ORF1b, 2803–4350 (1,548 bp); and ORF2, 4343–6691 (2,349 bp). The NCBI BLAST search revealed that the full sequence of Kor85 was the most similar to HAstV-8 (GenBank accession number: AF260508). ORF1a and ORF1b regions were 99.97% and 99.93% similar, respectively, to those of HAstV-2 strain isolated from Novosibirsk, Russia (KF039910). The ORF2 region of Kor85 was 99.96% similar to that of HAstV-8 strain isolated from Cuernavaca, Mexico (AF260508; MEGA version6.06). The sequence obtained in this study was registered at NCBI (KP862744).

Phylogenetic analysis

Phylogenetic analysis was performed to evaluate the genetic relationships among the HAstV-positive sample of our study and other published reference strains of HAstV. The complete nucleotide and amino acid sequences of Kor85 (KP862744) were highly similar (99.94% and 99.86%, respectively) to those of HAstV-8 and HAstV-2 (Figures 1 and 2). The nucleotide and amino acid sequences of Kor85 showed 99.64% and 99.65% similarity, respectively, to those of HAstV-7 (AF248738). The HAstV-2 and HAstV-8 sequences were found to be the most closely related to Kor85.

The nucleotide sequences of ORF1a and ORF1b of Kor85 were 99.97% similar and their amino acid sequences were 99.99% similar to those of HAstV-2; Figure 3A-3D). However, the nucleotide sequence of ORF2 of Kor85 was 99.96% similar and the amino acid sequence was 99.97% similar to those of HAstV-8; Figure 3E and 3F).

ORF analysis

The reference strains for comparing the full amino acid sequence were from the eight described genotypes reported from various countries, including the United States, Japan, Germany, Russia, and Mexico. The NCBI BLAST analysis suggested that the amino acid sequence of Kor85 showed the highest similarity (94%) to that of Mexico/2000/type8 strain (AF260508).

Among the amino acid sequences compared, only the Kor85 strain showed Pro89 in ORF2. The sequence comparison of Kor85 strain with HAstV-1 and HAstV-8 strains showed 99.86% to 99.94% identity with HAstV-2 and HAstV-8 strains. Kor85 and HAstV-2 ORF1a regions showed differences at 5 amino acids (AA33 Val → Ile, AA316 Ala → Ser, AA475 Glu → Gly, AA636 Asp → Glu, and AA922 Thr → Ile). The ORF1b region of Kor85 and HAstV-2 showed differences at 2 amino acid regions (AA154 Tyr → His and AA384 Ser → Arg). The ORF2 region of Kor85 and HAstV-8 showed differences for 4 amino acids (AA481 Ala → Met, AA553 Ile → Val, and AA577 Val → Ala). The sequence comparisons were performed using MegAlign (version 6.06).

Discussion

A wide genetic diversity has been reported in HAstV isolated from different geographic locations.

The comparison of the full sequence of Kor85 revealed that the eight serotypes had a common ancestor based on the sequence of HAstV-6 (HM237363) with the exception of HAstV-3 (AF141381) and HAstV-7 (AF248738). The Kor85 full sequence showed 99.94% similarity to the Mexico/2000/type8 strain (AF260508) [18,19].

The recombination event was noted in the ORF1b/ORF2 region in the Kor85 strain. It is known that being a crossover point in this region [11,20,21]. In 2008, ITA/2008/BA393/08-65 HAstV recombination strain was reported in Italy. The ORF1b region of this strain was similar to that of HAstV-1 and the ORF2 region was similar to that of HAstV-4. Further, this strain had a recombination site between ORF1b and ORF2.
Figure 3: Phylogenetic trees of the nucleotide sequences of HAstVs. The phylogenetic tree analysis based on the nucleotide (A) and amino acid sequence (B) of the ORF1a region, the nucleotide (C) and amino acid sequences (D) of the ORF1b region, the nucleotide (G) and amino acid sequences (F) of the ORF2 region of the HAstV Kor 85 (KP862744) and the reference strains.

Figure 4: SimPlot analysis of full sequences showed the similarities and the breakpoint among HAstV type2: KF039910 (blue line), HAstV type8: AF260508 (red line) and Kor85 (GenBank accession number KP962744) strains. The vertical axis indicates the similarity (%) of nucleotide sequence between the query strain and other reference strains. The horizontal axis indicates the positions of nucleotides. SimPlot analysis was performed using a window size or 200 nucleotides and 20 nucleotides step.
regions [22]. Similarly, in 2012, PR1365/2012/ITA/type3 recombinant strain was found in Italy; even in this strain, recombination was known to occur between ORF1b and ORF2 regions. The ORF1b region of this strain was similar to that of HAstV-1 and the ORF2 region was similar to that of HAstV-3 [23]. Furthermore, in Russia, recombination event occurred between ORF1b and ORF2 in the Rus-Nsc06-1029 recombinant strain. The ORF1b region of this strain was similar to that of HAstV-3 and ORF2 region was similar to that of the HAstV-2 genotype [24].

Therefore, studies on the genetic diversity and evolution of HAstV could provide important information for controlling human HAstV infection. In this study, the full-length sequence of a recombinant HAstV strain isolated from a clinical sample in South Korea was determined. This novel recombinant HAstV strain might be responsible for the severe HAstV outbreaks in Korea.

In conclusion, in this study, a recombinant strain of HAstV (with similarity to HAstV-2 and HAstV-8) was isolated from an acute enteritis patient. To our knowledge, this is the first report of the complete genome sequence of the recombinant strain from South Korea. We expect that other recombinant HAstV strains might be present, and guidelines need to be developed for identifying these new strains. Our data might also contribute to the development of HAstV vaccines based on the complete sequence of the recombinant strains isolated from South Korea.

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