Journal of Microbiology (2013) Vol. 51, No. 1, pp. 123–129
DOI 10.1007/s12275-013-2505-2

Full Sequence Analysis and Characterization of a Human Astrovirus Type 1 Isolate from South Korea

Sung-Geun Lee[1†], Lae-Hyung Kang[2†], Weon-Hwa Jheong[3], Mi-Hwa Oh[4], Gyu-Cheol Lee[5], Sujeong Park[3], and Soon-Young Paik[2*]

[1]Korea Zoonosis Research Institute, Chonbuk National University, Jeonju 561-756, Republic of Korea
[2]Department of Microbiology, College of Medicine, The Catholic University of Korea, Seoul 137-701, Republic of Korea
[3]Environmental Infrastructure Research Department, National Institute of Environmental Research, Incheon 404-708, Republic of Korea
[4]National Institute of Animal Science, Rural Development Administration, Suwon 414-706, Republic of Korea
[5]Water Analysis and Research Center, Korea Institute of Water and Environment, Korea Water Resources Corp., Daejeon 306-711, Republic of Korea

(Received September 14, 2012 / Accepted November 27, 2012)

Human astroviruses are recognized as an important cause of infantile gastroenteritis around the world. In South Korea, sporadic cases of HAstV infection have been reported since 2002. However, hitherto, there have been no studies reporting the whole genome sequence of an HAstV isolate from South Korea. Hence, we sequenced and analyzed the entire genome of an HAstV-1 strain (lhar) that was isolated in Seoul, South Korea. The whole-genome sequence analysis revealed 3 open reading frames comprising the whole genome: ORF1a (2,763 bp), ORF1b (1,548 bp), and ORF2 (2,364 bp). The lhar strain showed amino acid identities with 8 other reference strains of 87.6–98.7%, 94.2–98.8%, and 62.6–99.0% in the ORF1a, ORF1b, and ORF2 regions, respectively. The amino acid sequence of the capsid region encoded by ORF2 was compared with a total of 19 HAstV-1 strains and 8 HAstVs reference strains isolated in various countries. This revealed 1 amino acid substitution, at aa412 (Pro → Arg) in ORF2. This study, the first to report the full-length sequence of an HAstV isolated in South Korea, is meaningful in that it can be used as a full-length HAstV sequence standard for future comparison studies. It may also prove useful to the field of public health field by facilitating the diagnosis and the prediction of new emerging variants.

Keywords: Astrovirus, full genome sequence, phylogenetic tree, South Korea, Astrovirus type 1

[†]These authors contributed equally to this work.
*For correspondence. E-mail: paik@catholic.ac.kr; Tel.: +82-2-2258-7342; Fax: +82-2-535-6473

Introduction

Human astroviruses (HAstVs) are recognized as an important cause of infantile gastroenteritis around the world (Herrmann *et al.*, 1991). HAstV infections mainly cause watery diarrhea in children, the elderly, and immunocompromised individuals and less commonly cause fever, vomiting, anorexia, and abdominal pain (Moser and Schultz-Cherry, 2005; Finkbeiner *et al.*, 2008). The most commonly affected group includes children younger than 2 years. Transmission in children is usually person-to-person via the fecal-oral route. Symptoms manifest within 2 or 3 days postinfection and last for approximately the same amount of time (Moser and Schultz-Cherry, 2005). HAstV accounts for up to 10% of sporadic cases of nonbacterial diarrhea in children (Glass *et al.*, 1996; Kirkwood *et al.*, 2005; Klein *et al.*, 2006; Caracciolo *et al.*, 2007; Soares *et al.*, 2008). HAstVs are non-enveloped, of 28–30 nm in diameter, and contain a 6- to 8-kb positive-sense, single-stranded RNA genome. Three open reading frames (ORFs), ORF1a, ORF1b, and ORF2, have been identified in the genome. ORF1a encodes a serine protease, ORF1b encodes an RNA-dependent polymerase, and ORF2 encodes a capsid precursor protein (Mendez and Arias, 2007). Astrovirus capsid protein can be divided into three domains: a highly conserved N-terminal domain (S and P1 domain), a hypervariable domain (P2 domain), and a highly acidic C-terminal domain (Dong *et al.*, 2011). Eight genotypes of HAstV have been identified to date, HAstV-1 being the most commonly detected (Moser and Schultz-Cherry, 2005). In South Korea, sporadic cases of HAstV infections have been reported since 2002 (Jeong *et al.*, 2011).

According to a recent report, the HAstV infection rate in all age group was approximately 1% in South Korea in 2010 (http://www.cdc.go.kr/kcdchome). In our current study, the entire genomic sequence of a South Korean HAstV-1 (the predominant genotype) isolate was analyzed and compared with available reference strains to reveal the genetic relationship along the entire genome in South Korea.

Materials and Methods

Stool sample collection

An HAstV-positive stool sample was isolated from a 1-year-old female patient with acute gastroenteritis, in Seoul, South Korea, in February 2006. The sample was obtained from the Waterborne Virus Bank (Seoul, Korea). The stool sample was stored at −70°C.

Table 1. Primer sets used in this study

	Primer	Sequence (5′ → 3′)	Polarity	Region[a]	Reference
Diagnosis primer sets	Mon269	CAA CTC AGG AAA CAG GGT GT	+	4526–4545	Noel *et al.* (1995)
	Mon270	TCA GAA TGC ATT GTC ATT GGT	–	4955–4974	
Designed primer sets	ORF1a-1F	ATG GCA CAC GGT GAG CC	+	1–17	In this study
	ORF1a-1R	GTT TGC TAG AGC GAG CTT GAT T	–	612–633	
	ORF1a-2F	CAT TAA CAG AAA ACC TTG ACC TTA G	+	587–611	
	ORF1a-2R	GCC CTC ATA GCA CAC ATT CAC	–	1405–1425	
	ORF1a-3F	ATA AAA CCA GGT GCG TTA TGT G	+	1294–1315	
	ORF1a-3R	ATC TCA CGT TCC ATT GCA GTT	–	1923–1943	
	ORF1a-4F	GCT CAA AGA GGA AAT AGA GCG A	+	1785–1806	
	ORF1a-4R	GAA TTT GGC ATC ATC CTC ATC	–	2482–2502	
	ORF1a-5F	GGA ATC CTA TGA TTT TGA CTG G	+	2460–2481	
	ORF1a-5R	AAA ATT AGC AGG GAC GCA C	–	2805–2823	
	ORF1b-6F	TAC CAC TCA TTA GAT TCA TGG AAA TC	+	2751–2776	
	ORF1b-6R	CCA TCA TAG CGG GTC CAG	–	3500–3517	
	ORF1b-7F	GAA TAA ACA CTT CAT TGA ATT CGA	+	3476–3499	
	ORF1b-7R	GGG TGG TCT TCT GCC ATG	–	4100–4117	
	ORF2-8F	TAT CAG TTG CTT GCT GCG TT	+	4080–4099	
	ORF2-8R	GTG CTC CCA GTA GCG TCC T	–	4583–4601	
	ORF2-9F	CCT CAA CCC TGT CCT TGT TA	+	4563–4582	
	ORF2-9R	CAG TTC TAG TCC TCC CAG CA	–	5310–5329	
	ORF2-10F	GGT TGG TGG TTC GTA AAA CTT AT	+	5287–5309	
	ORF2-10R	GCT TCA TCG TCT TCG TCC TC	–	6295–6314	
	ORF2-11F	ACA GAT ACC GAC ATT GAG AGT ACA	+	6271–6294	
	ORF2-11R	GCT TCT GAT TAA ATC AAT TTT AAA TG	–	6661–6686	

[a] Each sequence number of the primer set region is indicated with the Beijing strain (FJ755402), except that of Mon269 and Mon270, which is indicated with the CAV4G strains (AF440797).

Viral nucleic acid extraction and reverse transcription-PCR (RT-PCR)

Viral genomic RNA was extracted from 140 ml of a 10% fecal suspension with the QIAamp Viral RNA Mini Kit (Qiagen, Germany) in accordance with the manufacturer's instructions.

For the detection of HAstV, reverse transcription polymerase chain reaction (RT-PCR) was performed with the OneStep RT-PCR Kit (Qiagen), with Mon 269 and Mon 270 primers based on the sequence of the astrovirus ORF2 region (Table 1). To facilitate the sequencing of the entire genome of the detected astrovirus strain, RT-PCR was performed with the OneStep RT-PCR Kit (Qiagen), with 11 pairs of newly designed primer sets (Table 1). Eleven fragments were amplified: 5 fragments for ORF1a, 2 fragments for ORF1b, and 4 fragments for ORF2. We used 5 ml of viral RNA as the template and 20 ml of the premixed kit solution. The PCR was carried out in a PCR System S1000^{TM} thermal cycler (Bio-Rad, USA) according to the following protocol: one initial RT step at 50°C for 30 min, followed by PCR activation at 95°C for 15 min; followed by 35 cycles of amplification at 30 sec at 94°C, 30 sec at 56°C, and 30 sec at 72°C; with a final extension step of 5 min at 72°C. The PCR products were then electrophoresed on a 1.5% agarose gel and stained with ethidium bromide.

Cloning and sequencing of RT-PCR products

The amplified fragments were purified from the gel using the HiYield Gel/PCR DNA Extraction Kit (RBC, Taiwan). These products were then cloned into the pGEM-T Easy vector (Promega, USA) according to the manufacture's recommendations with maximum incubation times and transformed into competent *E. coli*, DH5α cells (RBC). Transformants were selected on Luria-Bertani (LB) agar media (Duchefa, Netherlands) containing 50 μg/ml ampicillin. Clones were expanded overnight at 37°C in 10 ml LB media containing 50 μg/ml ampicillin, centrifuged at 4°C for 10 min at 800×g, resuspended in 600 ml fresh LB media with 10% glycerol, and stored at −80°C until further use. Plasmid DNA was purified using the HiYield^{TM} Plasmid mini kit (RBC) according to manufacturer's recommendations. DNA was sequenced by Cosmo Genetech (Korea).

Phylogenetic analysis

The sequence data analysis of the composite sequence of the 11 plasmids aligned via the Clustal W method using the DNAStar software (DNAStar Inc., USA) revealed that the entire genome was composed of 3 ORFs of 2,763 base pairs (bp; ORF1a), 1,548 bp (ORF1b), and 2,364 bp (ORF2). Dendrograms were constructed using the neighbor-joining method with MEGA software version 4.0 (Tamura *et al.*, 2007).

Similarity analysis

DNAStar software (DNAStar Inc., USA) was used to analyze the relationships among the aligned HAstV genome sequences. The genome sequences of lhra, HAstV-1 (GenBank accession nos. AY720892), HAstV-2 (GenBank accession no. L13745), HAstV-3 (GenBank accession no. AF141381),

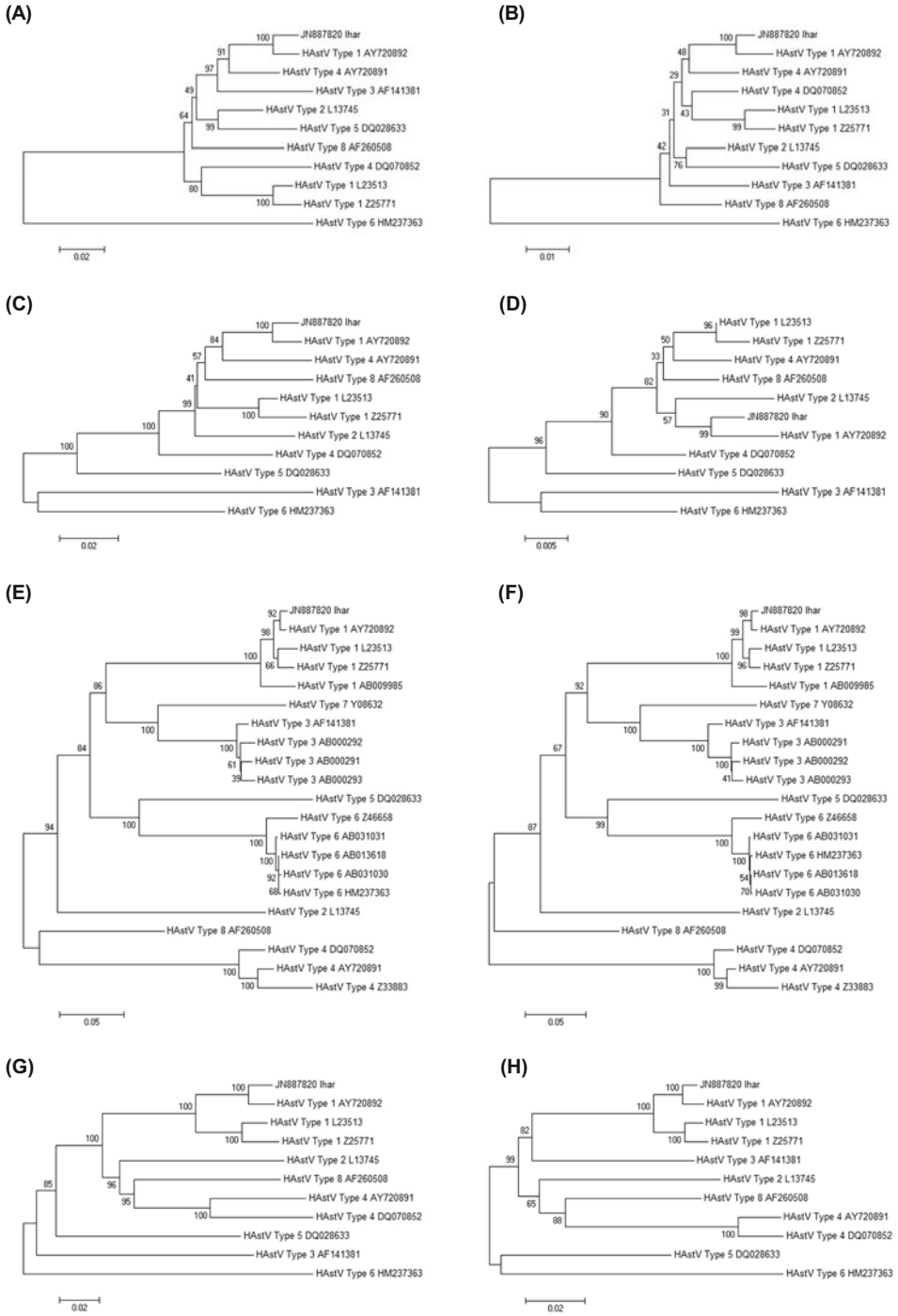

Fig. 1. Phylogenetic trees of the nucleotide sequences of the complete regions of HAstVs. The phylogenetic tree analysis based on the nucleotide (A) and amino acid sequences (B) of the ORF1a region, the nucleotide (C) and amino acid sequences (D) of the ORF1b region, the nucleotide (E) and amino acid sequences (F) of the ORF2 region, and the nucleotide (G) and amino acid sequences (H) of the complete region of the HAstV strain (lhar) and the reference strains.

HAstV-4 (GenBank accession nos. AY720891), HAstV-5 (GenBank accession no. DQ028633), HAstV-6 (GenBank accession nos. HM237363), HAstV-7 (GenBank accession nos. Y08632), and HAstV-8 (GenBank accession no. AF260508) were first aligned by using Clustal W of the MEGA software version 4.0, and then lhar was chosen as the query sequence for the similarity analysis.

Nucleotide sequence accession number

The nucleotide sequence of the HAstV-positive stool sample was isolates were submitted to GenBank database under the following accession numbers: JN887820.

Results

Phylogenetic analysis

To assess the genetic relationship between the lhar strain and the other reference strains isolated worldwide, the sequences of the ORFs and the whole genomes were subjected

to multiple sequence alignment analysis and phylogenetic analysis. With respect to ORF1a, the sequence comparisons revealed that the lhar strain shares the greatest identity with the HAstV-1 Dresden strain (AY720892), which was isolated from the Germany (97.8% for nucleotide and 98.7% for amino acid sequences, respectively). The lhar strain showed higher similarity (97.3%) to the HAstV-4 Dresden strain (AY720891) than to the HAstV-1 Oxford strain (L23513; 96.0%) at the amino acid level (Fig. 1B). The lhar strain was clustered with the HAstV-1 Dresden strain (AY720892) in a monophyletic branch (Figs. 1A and 1B). With respect to ORF1b, the sequence comparisons revealed that the lhar strain shares the greatest identity with the HAstV-1 Dresden strain (AY720892), which was isolated from the Germany (98.2% for nucleotide and 98.8% for amino acid sequences, respectively). The lhar strain showed higher similarity (98.3%) to the HAstV-4 Dresden strain (AY720891) than the HAstV-1 strain (Z25771; 97.9%) at the amino acid level (Fig. 1D). The lhar strain was clustered with the HAstV-1 Dresden strain (AY720892) in a monophyletic branch (Figs. 1C and 1D). With respect to ORF2, the sequence comparisons revealed that the lhar strain shares the greatest identity with the HAstV-1 Dresden strain (AY720892), which was isolated from the Germany (98.3% for nucleotide and 99.0% for amino acid sequences, respectively). Among the HAstV-1 strains, the lhar strain showed the lowest similarity (95.9%) to the J1050 strain (AB009985) at the amino acid level (Fig. 1F). The lhar strain was clustered with the HAstV-1 strains in a monophyletic branch (Figs. 1E and 1F). The entire genome sequence comparisons revealed that the lhar strain shares the greatest identity with the HAstV-1 Dresden strain (AY720892), which was isolated from the Germany (98.0% for nucleotide and 97.9% for amino acid sequences, respectively). The lhar strain was clustered with the HAstV-1 strains

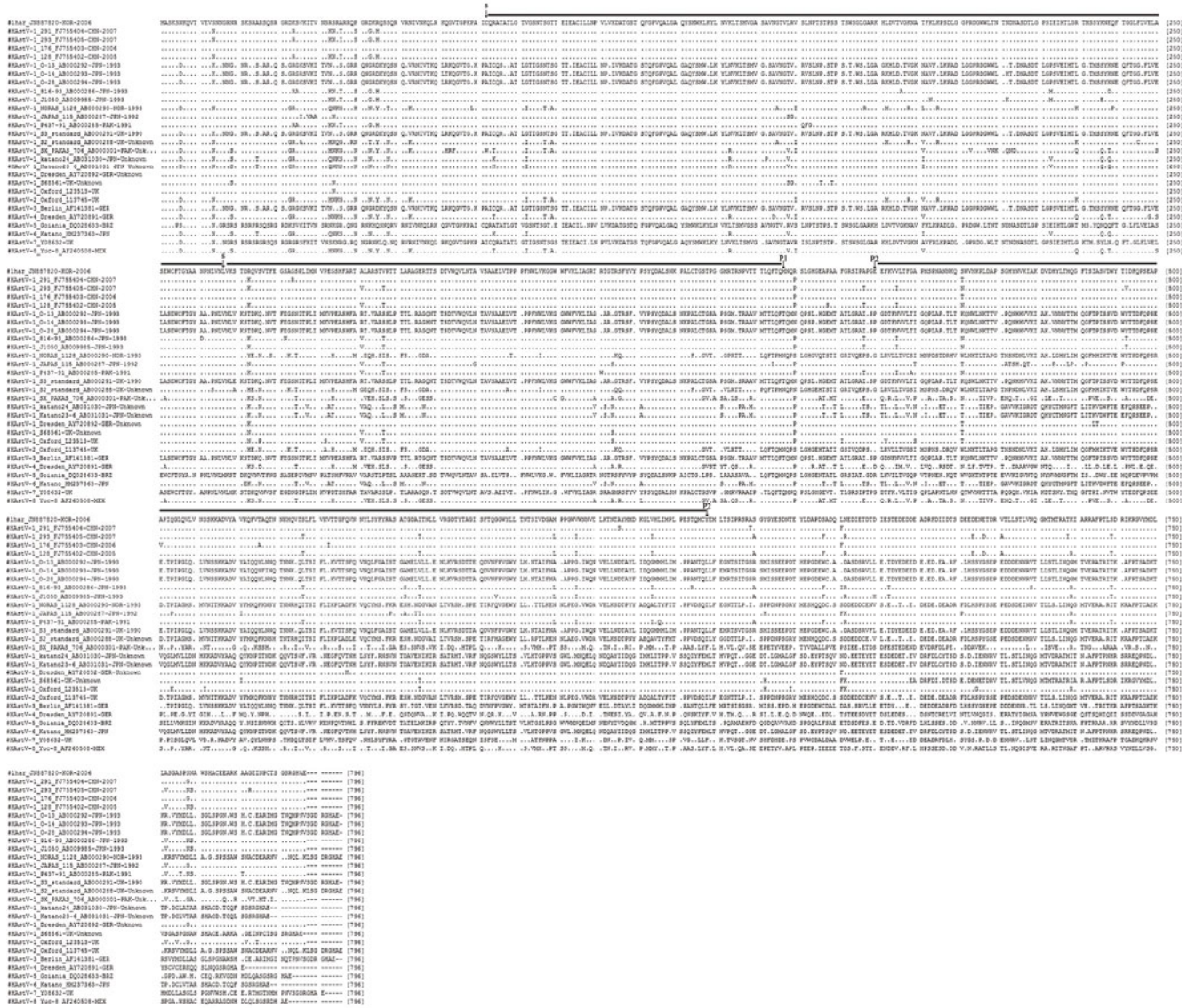

Fig. 2. **Alignment analysis for the ORF2 complete region of the HAstV strains.**

at the amino acid level (Figs. 1G and 1H). However, the lhar strain showed the highest similarity (97.9%) to the HAstV-1 Dresden strain (AY720892), whereas the HAstV-1 Oxford strain (L23513) and another HAstV-1 strain (Z25771) showed relatively low similarity (93.5–93.8%) at the amino acid level (Fig. 1H).

ORF analysis

The amino acid sequences of the entire capsid region encoded by ORF2 were compared with a total of 19 HAstV-1 strains and 8 HAstVs reference strains from the eight described genotypes reported from various countries, including the United States, the UK, Japan, China, and Germany. We detected 1 amino acid substitution (aa410, Pro → Arg) in ORF2. Among the HAstV-1 strains, the lhar strain showed the highest similarity (99.1%) to the Beijing/176/2006/CHN strain (FJ755403). In addition, only the lhar strain and the 176 strain showed as Arg 273 in ORF2. The sequence comparisons revealed that the lhar strain showed 95.8% and 98.0% identity with the 291 and 293 strains, respectively, which were both isolated in China in 2007. The lhar strain showed a higher similarity range (95.0–95.6%) with respect to the Aichi strains (816/93 and J1050) than did the Ehime strains (O-13, O-14, and O-28; 75.5–75.9%), which were isolated in Japan in 1993 (Fig. 2).

Similarity analysis

The 5′NTR of the HAstV-1 lhar strain showed 69.7–98.7% nucleotide sequence identity with those of genotypes 1 through 6 and genotype 8. Only 1 genotype (HAstV-6) among the reference strains showed the lowest identity (69.7%) at the nucleotide level. The genotypes of strains 1 and 4 showed the highest nucleotide sequence identity (98.7%). ORF1a of the HAstV-1 lhar strain showed 80.1–97.8% nucleotide sequence identity and 87.6–98.7% amino acid sequence identity with those of genotypes 1 through 6 and genotype 8. All genotypes among the reference strains showed lower identity (87.3–97.3%) than the HAstV-1 strain (98.7%) at the amino acid level. Unlike ORF1a, ORF1b exhibited higher nucleotide (83.6–98.2%) and amino acid (94.2–98.8%) sequence identity with that of genotypes 1 through 6 and genotype 8, indicating that ORF1b is more conserved among the lhar strain and the HAstVs genotypes than ORF1a. ORF2 of the HAstV-1 lhar strain showed 63.2–98.3% nucleotide identity and 62.6–99.0% amino acid identity with that of genotypes 1 through 8. All of the genotypes generally showed a low identity range (lower than 80%), except for the HAstV-1 genotype. The 3′NTR of the HAstV-1 lhar strain showed 90.0–100% nucleotide sequence identity with those of genotypes 1 through 8. All of the genotypes showed a high identity range (higher than 90%). The genotype 1 strains among the all genotypes showed the highest nucleotide sequence identity (100%; Table 2).

Discussion

HAstV is recognized as a common cause of infantile acute gastroenteritis worldwide. HAstV, along with rotaviruses, adenovirus, and noroviruses, are considered important viral agents of acute gastroenteritis in South Korea (Kim *et al*., 1996; Park *et al*., 2010). In human, eight genotypes have been described, which have been associated with up ~10% sporadic cases of nonbacterial diarrhea in children and 0.5–15% outbreaks (Glass *et al*., 1996; Akihara *et al*., 2005; Kirkwood *et al*., 2005; Klein *et al*., 2006; Caracciolo *et al*., 2007; Svraka *et al*., 2007; Soares *et al*., 2008; Lyman *et al*., 2009).

The Korean Center for Disease Control and Prevention, in collaboration with 16 laboratories of local Public Health Institutes and more than 100 sentinel hospital participants, initiated a viral agent surveillance system for acute gastroenteritis in 1999. Data from 2002 to 2007 showed that HAstV accounted for 2,057 (1.3%) (detection rate per year, 0.6–2.4%) of 160,027 patients with gastroenteritis in South Korea (Jeong *et al*., 2011). Consistent with previous reports, the HAstV infection rate was approximately 1% in South Korea in 2010 (http://www.cdc.go.kr/kcdchome). The HAstV-1 among the 8 genotypes was the dominant genotype in every year since 2002 in South Korea (Jeong *et al*., 2011). Previous studies have reported that HAstV-1 is also the predominant HAstV genotype in Egypt, Italy, France, China, and Japan (Medina *et al*., 2000; Naficy *et al*., 2000; Guix *et al*., 2002; Galdiero *et al*., 2005; Liu *et al*., 2007).

Our phylogenetic analysis suggests that HAsV-6 may be an ancestor of other HAstV genotypes as shown by the phylogenetic analysis of the entire genome sequence (Fig. 1G). This obserbation was further supported by the phylo-

Table 2. Nucleotide and amino acid sequence identity between the HAstV-1 lhar strain and the full-length sequences of various HAstV reference strains

Genotype (accession no.)	Percent identity							
	5′NTR	ORF1a		ORF1b		ORF2		3′NTR
	Nucleotide	Nucleotide	Amino acid	Nucleotide	Amino acid	Nucleotide	Amino acid	Nucleotide
HAstV-1 (AY720892)	**98.7**	**97.8**	**98.7**	**98.2**	**98.8**	**98.3**	**99.0**	**100**
HAstV-2 (L13745)	89.5	93.2	97.0	93.5	98.1	66.8	69.5	93.8
HAstV-3 (AF141381)	96.1	92.8	96.4	83.6	94.2	70.6	77.0	95.0
HAstV-4 (AY720891)	98.7	94.9	97.3	94.6	98.3	63.2	62.6	90.0
HAstV-5 (DQ028633)	82.4	91.7	95.4	88.5	96.1	68.1	69.0	96.2
HAstV-6 (HM237363)	69.7	80.1	87.6	85.7	94.8	69.3	71.0	97.5
HAstV-7 (Y08632)	NA	NA	NA	NA	NA	69.4	73.3	93.8
HAstV-8 (AF260508)	89.5	91.7	96.3	93.3	98.3	69.8	70.1	97.5

NA, Not available.
The values in bold indicates the highest sequence identity rate.

genetic analysis of the ORF1a (Figs. 1A and 1B). In addition, the analysis of HAstVs ORF2 suggests that J1050 strain (AB009985) isolated in Japan in 1993 may have been the common ancestor of other HAstV-1 genotypes. Morever, detailed Phylogenetic analysis indicated that HAstV-1 strain could be classified into four lineages (1a-1d), with the lhar strain isolated in 2006 clustered into lineage 1d (data not shown). In 2002, 91.67% of HAstV-1 strains were type 1a although this prevalence significantly decreased from year to year, reaching 33% in 2007, whereas the HAstV-1d was first detected in 2003 and exhibited a peak prevalence of 26.67% in South Korea in 2006 (Mendez and Arias, 2007). However, Globally HAstV-1d is currently the most prevalent.

Consistent with previous reports of other HAstV genotypes, our results also show the existence of 3 potential cleavage sites as Lys 71, Arg 361, and Arg 395 in HAstV-1 ORF2. It is thought that the cleavage at Lys 71 leads to the generation of the 79-kDa capsid protein. The 79-kDa capsid protein can be converted into three smaller peptides–VP34, VP29, and VP26–and leads to an enhancement of HAstV infectivity (Bass and Qiu, 2000; Méndez-Toss *et al.*, 2000; Liu *et al.*, 2007). Our observations support the critical role of these three amino acid residues in HAstV replication and pathogenesis.

The analysis of the S domain of the lhar strain revealed high similarity (91.9–96.8%) to the 7 reference strains (HAstV-2 to HAstV-8), whereas the P1 domain showed a relatively low similarity range (76.6–87.9%). In the case of the P2 domain, the lhar strain showed the lowest similarity (44.7–62.0%).

As for P2, the lhar strain showed 98.2% amino acid similarity with Beijing/176/2006/CHN strain isolated in 2006, except for S and P1 domain (100% amino acid similarity). This result showed that HAsV-1 of common an ancestor may be co-circulated in both South Korea and China in 2006.

In our study, we found amino acid substitution in ORF1a and ORF1b. The data from 944 amino acid sequence analyses showed 3 amino acid substitutions in ORF1a (data not shown): aa620 (Gln or Pro → His), aa630 (Ser or Asn → Gly), and aa787 (Gln → Lys). The data from 515 amino acid sequence analyses showed 2 amino acid substitutions in ORF1b (data not shown): aa22 (Ala → Ser) and aa246 (Gly → Val).

This is the first study reporting the full-length sequence of an HAstV-1 isolated in South Korea from a clinical sample. We suggest that this sequence will be useful for comparisons with the full-length HAstV-1 sequences of other strains identified globally in the future. The information acquired from the whole-genome sequencing undertaken in this study may prove useful not only for more accurate diagnoses of HAstV but also for basic research relating to the elucidation of genetic functions. Furthermore, it may prove useful for the prediction of newly appearing pandemic variants via comparison with HAstVs in neighboring countries, in fundamental research for vaccine development, and eventually, in the field of public health, with the provision of new emerging strains of HAstV.

This study, the first to report the full-length sequence of an HAstV isolated in South Korea, is meaningful in that it can be used as a full-length HAstV sequence standard for future comparison studies. It may also prove useful to the field of public health field by facilitating the diagnosis and the prediction of new emerging variants.

Acknowledgements

This study was supported by the 2012 Agenda R&D Program (grant no. PJ007586) of the Rural Development Administration and Basic Science Research Program (2012-0000328) through NRF grant funded by the MEST.

References

Akihara, S., Phan, T.G., Nguyen, T.A., Hansman, G., Okitsu, S., and Ushijima, H. 2005. Existence of multiple outbreaks of viral gastroenteritis among infants in a day care center in Japan. *Arch. Virol.* **150**, 2061–2075.

Bass, D.M. and Qiu, S. 2000. Proteolytic processing of the astrovirus capsid. *J. Virol.* **74**, 1810–1814.

Caracciolo, S., Minini, C., Colombrita, D., Foresti, I., Avolio, M., Tosti, G., Fiorentini, S., and Caruso, A. 2007. Detection of sporadic cases of Norovirus infection in hospitalized children in Italy. *New Microbiol.* **30**, 49–52.

Dong, J., Dong, L., Méndez, E., and Tao, Y. 2011. Crystal structure of the human astrovirus capsid spike. *Proc. Natl. Acad. Sci. USA* **108**, 12681–12686.

Finkbeiner, S.R., Kirkwood, C.D., and Wang, D. 2008. Complete genome sequence of a highly divergent astrovirus isolated from a child with acute diarrhea. *Virol. J.* **5**, 117.

Galdiero, E., Marinelli, A., Pisciotta, M.G., Pagliara, I., Di Monteforte, E.S., and Liguori, G. 2005. Reverse transcriptase-PCR for the detection of Astrovirus in children with nosocomial diarrhea in Naples, Italy. *Med. Mal. Infect.* **35**, 213–217.

Glass, R.I., Noel, J., Mitchell, D., Herrmann, J.E., Blacklow, N.R., Pickering, L.K., Dennehy, P., Ruiz-Palacios, G., de Guerrero, M.L., and Monroe, S.S. 1996. The changing epidemiology of astrovirus-associated gastroenteritis: a review. *Arch. Virol.* Suppl **12**, 287–300.

Guix, S., Caballero, S., Villena, C., Bartolomé, R., Latorre, C., Rabella, N., Simó, M., Bosch, A., and Pintó, R.M. 2002. Molecular epidemiology of astrovirus infection in Barcelona, Spain. *J. Clin. Microbiol.* **40**, 133–139.

Herrmann, J.E., Taylor, D.N., Echeverria, P., and Blacklow, N.R. 1991. Astroviruses as a cause of gastroenteritis in children. *N. Engl. J. Med.* **324**, 1757–1760.

Jeong, A.Y., Jeong, H.S., Jo, M.Y., Jung, S.Y., Lee, M.S., Lee, J.S., Jee, Y.M., Kim, J.H., and Cheon, D.S. 2011. Molecular epidemiology and genetic diversity of human astrovirus in South Korea from 2002 to 2007. *Clin. Microbiol. Infect.* **17**, 402–408.

Kim, H.J., Yang, J.M., and Kim, K.H. 1996. Genome type analysis of human adenoviruses associated with pediatric gastroenteritis in Korea. *J. Bacteriol. Virol.* **26**, 23–30.

Kirkwood, C.D., Clark, R., Bogdanovic-Sakran, N., and Bishop, R.F. 2005. A 5-year study of the prevalence and genetic diversity of human caliciviruses associated with sporadic cases of acute gastroenteritis in young children admitted to hospital in Melbourne, Australia (1998–2002). *J. Med. Virol.* **77**, 96–101.

Klein, E.J., Boster, D.R., Stapp, J.R., Wells, J.G., Qin, X., Clausen, C.R., Swerdlow, D.L., Braden, C.R., and Tarr, P.I. 2006. Diarrhea etiology in a children's hospital emergency department: A prospective cohort study. *Clin. Infect. Dis.* **43**, 807–813.

Liu, M,Q., Yang, B.F., Peng, J.S., Zhou, D.J., Tang, L., Wang, B., Liu, Y., Sun, S.H., and Ho, W.Z. 2007. Molecular epidemiology of astrovirus infection in infants in Wuhan, China. *J. Clin. Microbiol.*

45, 1308–1309.

Lyman, W.H., Walsh, J.F., Kotch, J.B., Weber, D.J., Gunn, E., and Vinje, J. 2009. Prospective study of etiologic agents of acute gastroenteritis outbreaks in child care centers. *J. Pediatr.* **154**, 253–257.

Medina, S.M., Gutierrez, M.F., Liprandi, F., and Ludert, J.E. 2000. Identification and type distribution of astroviruses among children with gastroenteritis in Colombia and Venezuela. *J. Clin. Microbiol.* **38**, 3481–3483.

Mendez, E. and Arias, C.F. 2007. Astroviruses: The astroviruses. Fields virology. *In* Knipe, D.M. and Howley, P.M. (eds.) 5th ed., vol. 1, pp. 981–1000. Lippincott/The Williams and Wilkins, Philadelphia, USA.

Méndez-Toss, M., Romero-Guido, P., Munguía, M.E., Méndez, E., and Arias, C.F. 2000. Molecular analysis of a serotype 8 human astrovirus genome. *J. Gen. Virol.* **81**, 2891–2897.

Moser, L.A. and Schultz-Cherry, S. 2005. Pathogenesis of astrovirus infection. *Viral. Immunol.* **18**, 4–10.

Naficy, A.B., Rao, M.R., Holmes, J.L., Abu-Elyazeed, R., Savarino, S.J., Wierzba, T.F., Frenck, R.W., Monroe, S.S., Glass, R.I., and Clemens, J.D. 2000. Astrovirus diarrhea in Egyptian children. *J. Infect. Dis.* **182**, 685–690.

Noel, J.S., Lee, T.W., Kurtz, J.B., Glass, R.I., and Monroe, S.S. 1995. Typing of human astroviruses from clinical isolates by enzyme immunoassay and nucleotide sequencing. *J. Clin. Microbiol.* **33**, 797–801.

Park, J.K., Suh, C.I., Lee, N.S., Lee, S.G., Cheon, D.S., Oh, M.H., Oh, S.H., Park, J.W., and Paik, S.Y. 2010. Antibody production of baculovirus-expressed VP6 from porcine group C rotavirus. *J. Bacteriol. Virol.* **40**, 83–89.

Soares, C.C., Maciel de Albuquerque, M.C., Maranhao, A.G., Rocha, L.N., Ramirez, M.L., Benati, F.J., Timenetsky Mdo, C., and Santos, N. 2008. Astrovirus detection in sporadic cases of diarrhea among hospitalized and non-hospitalized children in Rio De Janeiro, Brazil, from 1998 to 2004. *J. Med. Virol.* **80**, 113–117.

Svraka, S., Duizer, E., Vennema, H., de Bruin, E., van der Veer, B., Dorresteijn, B., and Koopmans, M. 2007. Etiological role of viruses in outbreaks of acute gastroenteritis in The Netherlands from 1994 through 2005. *J. Clin. Microbiol.* **45**, 1389–1394.

Tamura, K., Dudley, J., Nei, M., and Kumar, S. 2007. MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. *Mol. Biol. Evol.* **24**, 1596–1599.